ICLUSIG®: Uncover a DEEP RESPONSE

As a result, 23% of chronic phase CML patients achieved a deep molecular response (MR4.5) with Iclusig®.1, 2 Iclusig® is the 3rd generation TKI with the potential to deliver FAST, DEEP and DURABLE response in patients with concern with CML.1-3

Este medicamento no se encuentra comercializado en España.

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Dosage and administration:
Recommended starting dose 45 mg once daily; swallow tablets whole. Assess and actively manage cardiovascular and haematological effects. Assess hepatic enzymes, LFTs peripheral oedema, rash, dry skin, pain incl. back, bone & joint pain, lower limb oedema, dyspepsia, vomiting, fatigue, arthralgia, myalgia, muscle spasms, abdominal pain, nausea, constipation, abdominal distension, flushing, diarrhoea, dysphagia, dermatitis, dry mouth, increased liver enzymes, hepatitis, jaundice, pancreatitis. At 4 years, 23% of chronic-phase CML patients achieved a deep molecular response (MR4.5) with Iclusig®.1-3

---

In case of disease progression or severe adverse reactions consider dose reduction to 15 mg for CP-CML patients who achieve a Major Cytogenetic Response; consult the SmPC for full details of all recommended dose modifications. Contraindications: Myelosuppression: Perform Full Blood Count every 2 weeks for the first 3 months and then monthly as clinically indicated. Venous thromboembolism: Perform an initial test at baseline. Iclusig® is the 3rd generation TKI with the potential to deliver FAST, DEEP and DURABLE response in patients with concern with CML.1-3

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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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YoungEHA Best Abstract Awards

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

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- Chronic myeloid leukemia - Clinical 2
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- Gene therapy, cellular immunotherapy and vaccination
- Indolent Non-Hodgkin lymphoma - Clinical
- Infectious diseases, supportive care
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- Myeloma and other monoclonal gammopathies - Clinical 3
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- Acute lymphoblastic leukemia - Clinical
- Acute myeloid leukemia - Biology
- Acute myeloid leukemia - Clinical
- Aggressive Non-Hodgkin lymphoma - Clinical
- Bleeding disorders (congenital and acquired)
- Bone marrow failure syndromes incl. PNH - Clinical
- Chronic lymphocytic leukemia and related disorders - Biology
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- Chronic myeloid leukemia - Clinical
- Enzymopathies, membranopathies and other anemias
- Gene therapy, cellular immunotherapy and vaccination
- Hematopoiesis, stem cells and microenvironment
- Hodgkin lymphoma - Clinical
- Indolent Non-Hodgkin lymphoma - Clinical
- Infectious diseases, supportive care
- Iron metabolism, deficiency and overload
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- Myeloproliferative neoplasms - Clinical
- Non-Hodgkin & Hodgkin lymphoma - Biology
- Other Non-malignant hematopoietic disorders
- Platelets disorders
- Quality of life, palliative care, ethics and health economics
- Sickle cell disease
- Stem cell transplantation - Clinical
- Stem cell transplantation - Experimental
- Thalassemias
- Thrombosis and vascular biology
- Transfusion medicine
A complete session overview is available via the mobile app or the online program at ehaweb.org
**BACKGROUND:** Cytogenetic risk status in multiple myeloma (MM) studies is traditionally determined by using fluorescence in situ hybridization (FISH) or karyotyping to assess chromosomal abnormalities. However, these technologies have limited resolution and a narrow target range, and reproducible interpretation may be confounded by inter-laboratory variation.

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

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

**Results:** Based on the RNA-Seq and exome results, cytogenetic risk status in the CASTOR and POLLUX studies was defined as high risk with either t(4;14), t(14;16), or del17p, and standard risk with the confirmed absence of these molecular abnormalities. Comparisons of NGS with FISH showed high concordance for t(4;14), t(14;16), and del17p in both studies (Table 1).

**Summary/Conclusions:** These studies represent the first, comprehensive use of NGS in global phase 3 clinical trials in RRMM. The NGS methodology accurately identified the presence of defined risk populations t(4;14), t(14;16), and del17p and showed good concordance with FISH. As FISH was performed locally with different probes and pathologists, the high degree of concordance between FISH and NGS is notable and supports the use of NGS for determining cytogenetic risk in patients with RRMM. The utility of NGS in these clinical studies extends far beyond the detection of cytogenetic abnormalities and additional analysis are planned to interrogate these datasets in the identification of novel biomarkers.

**Figure 1.**

**Table 1.**

<table>
<thead>
<tr>
<th>Concordance rate between FISH and NGS</th>
<th>POLLUX</th>
<th>CASTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14)</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>96%</td>
<td>97%</td>
</tr>
<tr>
<td>del17p</td>
<td>98%</td>
<td>99%</td>
</tr>
</tbody>
</table>
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 among del17, t(14;16) or t(4;14); 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 the recruitment, patients took the same chemotherapy to identify they were refractory to cyclophosphamide monotherapy) and engineered-T cells were reinfused on day 0, 2, and 6. This trial was divided into the dose escalation stage and expansion cohort. Toxicity and responses were assessed according to the Common Terminology Criteria for Adverse Events (version 4.0) and International Myeloma Working Group (IMWG) Uniform Response Criteria for Multiple Myeloma, respectively.

**Results:** As of the February 20th, 2017 data cut-off, 22 patients had been enrolled, two of whom were diagnosed as plasma cell leukemia. The male:female ratio was 11:11 and median age was 53.5 years. Chromosomal abnormalities were detectable by FISH in eight patients, two of whom involved in the deficiency of p53. Eleven patients were triple refractory (chemotherapy, proteasome inhibitors, and immunomodulatory drugs), 11 resisted to double prior treatments (chemotherapy and proteasome inhibitors/ immunomodulatory drugs), and four relapsed after autologous hematopoietic stem cell transplant. The median number of infused CAR T cells was 4.0×10^6 (range, 1.5×10^6-7.0×10^6) per kg. The median follow-up was 131.5 (range, 29-327) days. 100% of patients achieved an objective response. The first six patients achieved complete responses with flow MRD-negative; 14 patients achieved very good partial responses; one patient, with renal failure, achieved partial response; all these 22 patients had kept their best response at the end of follow-up. The pictures we enclosed were the subcutaneous nodules in one patient with extramedullary plasmacytoma. We found that the nodules were obviously decreased after the infusion and disappeared finally. Another one achieved transient partial response, which last for 12 days. He then took the secondary infusion but failed since the post-operation large-cutaneous nodules was cytokine release syndrome (CRS). Toxicities were minimal except for two grade 3 CRS and one grade 4 CRS. All CRSs were controllable with nonspecific measures.

**Summary/Conclusions:** Our findings demonstrated the safety and antimyeloma 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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**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, 8, 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 CRS/GPR/PR after PCD: 47.1/66.7/42.9% [cardiac] and 68.8/63.6/62.5% [renal]; P>0.05). Similarly, time or depth of patients’ last HR did not impact the NEO001 organ response rate (P>0.05). Patients with NEO001 organ responses were no more likely to have had their last PCD therapy <6 than 6 months from their first NEO001 dose. Patients’ previous PCD treatment type was not related to the corticosteroid for spinal meningioma. He terminally died 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 nonspecific measures (NSAIDs) or tocilizumab and no dose-limiting toxicities or treatment-related deaths were observed (Figure 1).

**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.
Agnogenic Non-Hodgkin lymphoma - 1st line

S105

RITUXIMAB MAINTENANCE AFTER AUTOLOGOUS TEM CELL TRANSPLANTATION PROLONGS SURVIVAL IN YOUNGER PATIENTS WITH MALT CELL LYMPHOMA: FINAL RESULTS OF THE LYMA TRIAL OF THE EUKLID GROUP


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

Aims: We investigated whether or not rituximab maintenance (RM) could be an acceptable option to improve response rates at the end of treatment. The majority of the patients in this trial represented a poor prognosis group by age and IPI. In this context, treatment response to this regimen may warrant further exploration.

Methods: Five pts of the dose escalation portion and 40 pts of the expansion portion completed RM for 3 years after ASCT. The following reasons: Gr 5 atrial fibrillation (after C2, not attributed to pola by investigator), E. coli UTI (C5), worsening essential tremor (C3), PN (C7). During follow-up, median study duration of 9.5 months, (range 1.3-28 months), only 1 pt had a disease progression in follow up.

Summary/Conclusions: RM at 1.8 mg/kg in combination with R-CHP in 1L patients is an acceptable option to improve response rates at the end of treatment. The majority of the patients in this trial represented a poor prognosis group by age and IPI. In this context, treatment response to this regimen may warrant further exploration.

References
1. 0.072 to 0.971) for RM (n=120) vs 0.193 to 0.750) for observation (p=0.0413). The death rate was lower for patients in the RM arm was significantly lower (hazard ratio (HR)=0.5; 95%CI, 0.255 to 0.986) than for patients in the observation arm.

S106

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

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Background: Polatubazum vedotin (pola) is an antibody drug conjugate containing the anti-mitotic MMAE targeting CD79b, an antigen expressed ubiquitously in DLBCL. Pola as monotherapy and in combination with anti-CD20 antibodies demonstrated encouraging efficacy in r/r DLBCL. The initial dose-escalation portion of this multicenter, open-label Ph I/Ib study of pola in combination with rituximab, cyclophosphamide, doxorubicin, and prednisone (pola-R-CHP) showed an acceptable safety profile and established a recommended Ph II dose of pola at 1.8 mg/kg. We report updated safety and efficacy results for the Ph II dose in 45 previously untreated DLBCL patients (pts) (ClinicalTrials.gov, NCT01992653).

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

Methods: Five pts of the dose escalation phase and the 40 pts of the expansion phase were included in this analysis. All pts provided informed consent to partic- ipate in the study. All had newly diagnosed DLBCL and were treated with pola at 1.8 mg/kg and R-CHP at standard doses every 21 days for 6 or 8 cycles. Investigator assessments for anti-tumor activity were performed according to IWG 2007 following 4 cycles and at the end of study treatment (EOT).

Results: All 45 pts received at least one dose of study drug. The median age was 69 years; 93% were >60 years, 33% ECOG >1, 82% Stage III/IV, and 78% IPI 3-5. Of the 29 pts with cell of origin (COO) status by digital gene expression, 11 (38%) were ABC, 14 (48%) were GC, while 4 (14%) were unclassified. Forty patients completed 6 or 8 cycles (23 and 17 pts respectively). All pts experienced at least one AE. Grade (G) 3/4 AEs occurred in 58%, and one pt experienced a G 5 atrial fibrillation. G 3/4 neutropenia and febrile neutropenia (FN) occurred in 27% and 11%, Serious adverse events (SAEs) were reported in 17 pts (38%) including 3 FN, and 2 each of neutropenia, pneumonia, pulmonary embolism and influenza. Peripheral neuropathy (PN) occurred in 18 (40%) pts. Among patients with RM vs 12 pts, 1 were G 2, and 2 were G 3. All Gr 2/3 PN and febrile neutropenia occurred in 27% and 11%. Rates of neutropenia, pneumonia, pulmonary embolism and influenza were similar in the 2 arms. PN was the most common AE, occurring in 27% and 11%. Serious adverse events (SAEs) were reported in 17 pts (38%) including 3 FN, and 2 each of neutropenia, pneumonia, pulmonary embolism and influenza. Peripheral neuropathy (PN) occurred in 18 (40%) pts. Among patients with RM vs 12 pts, 1 were G 2, and 2 were G 3. All Gr 2/3 PN and febrile neutropenia occurred in 27% and 11%. Rates of neutropenia, pneumonia, pulmonary embolism and influenza were similar in the 2 arms. PN was the most common AE, occurring in 27% and 11%. Rates of neutropenia, pneumonia, pulmonary embolism and influenza were similar in the 2 arms.
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>Endpoint</th>
<th>Rituximab SC plus CHOP</th>
<th>Rituximab IV plus CHOP</th>
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<tbody>
<tr>
<td>No. of patients with CR</td>
<td>342</td>
<td>342</td>
</tr>
<tr>
<td>CR rate (%)</td>
<td>50.6 (95% CI 35.9–65.9)</td>
<td>42.4 (95% CI 34.9–50.7)</td>
</tr>
<tr>
<td>PR</td>
<td>313 (73.8–43.8)</td>
<td>313 (73.8–43.8)</td>
</tr>
<tr>
<td>SD</td>
<td>110 (26.2–45.9)</td>
<td>113 (26.2–46.8)</td>
</tr>
<tr>
<td>PR or ND</td>
<td>822 (76.4–88.6)</td>
<td>778 (74.6–81.9)</td>
</tr>
<tr>
<td>14 days</td>
<td>40 (3.6–7.5)</td>
<td>43 (3.6–7.5)</td>
</tr>
<tr>
<td>30 days</td>
<td>45 (4.1–7.6)</td>
<td>47 (4.1–7.6)</td>
</tr>
<tr>
<td>60 days</td>
<td>48 (4.3–7.9)</td>
<td>49 (4.3–7.9)</td>
</tr>
<tr>
<td>90 days</td>
<td>46 (4.2–7.5)</td>
<td>47 (4.2–7.5)</td>
</tr>
<tr>
<td>180 days</td>
<td>49 (4.5–8.0)</td>
<td>49 (4.5–8.0)</td>
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</table>

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

Methods: Fresh frozen DNA from diagnostic bone marrow samples from MCL2 and MCL3 were analyzed. In both trials, patients received intensified first-line induction therapy with alternating courses of R-CHOP and R-hd-Cytarabine and consolidation with high-dose therapy and ASCT. (Geissler 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%.

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.

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

Results: Analytical validation of the MyHEME panel established an average read depth of 1,175x (with a median read depth of 1,088x) for the DNA targets and an average transcripts per million (TPM) of 2,256 (with a median TPM of 743) for the RNA targets. For the DNA targets, we established sensitivity >95% (99.8% for SNVs at a 2.5% limit of detection (LOD); 100% for coding indels at a 5.0% 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-confinned when observed in both the DNA and RNA targets. For example, we identified a novel t(9;22) translocation causing a NUP214-XXR3 gene fusion using both the DNA and RNA targets. Additionally, while RNA data provides the fused exons of the transcripts, DNA data gives the precise genomic breakpoint coordinate.

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

S109

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

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

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

Methods: Fresh frozen DNA from diagnostic bone marrow samples from MCL2 and MCL3 were analyzed. In both trials, patients received intensified first-line induction therapy with alternating courses of R-CHOP and R-hd-Cytarabine and consolidation with high-dose therapy and ASCT. (Geissler 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%.

Results: We investigated the presence of CDKN2A and TP53 deletions in diagnostic samples from 175 and 157 patients, respectively. Patients were treated for 9 months and <6 years (median, 58 range, 37-65). Fifty-three percent were either MIPI intermediate- or high-risk, 17% had blastoid morphology and 42% had Ki67>30%, and 83% had bone marrow involvement at diagnosis. After a median follow-up of 9.2 years, median overall (OS), progression-free survival (PFS) and cumulated incidence of relapse (CIR) of all patients were 12.4 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<0.001 for all three outcomes), respectively. Preliminary analysis of all TP53 mutations in 29 patients revealed that 38% were coding deletions. 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>30%, NOTCH1 mutations, TP53 mutations, ALL- and delt-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 GILTERITINIB IMPROVES SURVIVAL IN FLT3 MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKAEMIA


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

Methods: This exploratory analysis evaluated molecular response in patients aged ≥18 years with FLT3-ITDmut+r/r AML who had been treated with 120 or 200 mg/d gilteritinib. These doses were identified as their ability to induce high clinical response rates, and consistent, potent FLT3 inhibition in correlative assays. Molecular response was assessed in patients who had bone marrow aspirates obtained at baseline and at ≥1 additional time point. FLT3-ITD and total FLT3 were quantified by next-generation sequencing to assess molecular response. A Cox regression model of OS by Kaplan-Meier estimation established a FLT3-ITD:total FLT3 ratio (ITD signal ratio) of 10−2 as the threshold for improved survival.

Results: Of the 147 FLT3-ITDmut+patients who had received gilteritinib 120 or 200 mg/d, 80 patients had bone marrow aspirates at baseline and at ≥1 additional time point. FLT3-ITD and total FLT3 were quantified by next-generation sequencing to assess molecular response. Of the 147 FLT3-ITDmut+patients who had received gilteritinib 120 or 200 mg/d, 80 patients had bone marrow aspirates at baseline and at ≥1 additional time point, and were included in this analysis. The composite response rate (defined as CR plus CRi plus PR) for these 80 patients was 55%. During response, 20 patients (25%) had an ITD signal ratio of ≤10−2. Of these 20 patients, 18 had an ITD signal ratio of ≤10−3 (major molecular response [MMR]) and 13 had an ITD signal ratio of ≤10−4 (minimal residual disease [MRD] negative). The median time to achieve minimum ITD signal ratio was 54 days.

Summary/Conclusions: Molecular responses to gilteritinib in FLT3-ITDmut+r/r AML correlated with clinical response and improved OS. This is the first demonstration of a robust molecular response to a FLT3 inhibitor in AML. These data suggest that the ITD signal ratio may predict a durable clinical benefit of gilteritinib therapy and validate FLT3 as a critical therapeutic target in AML.

S111

RISK-ADAPTED, MRD-DIRECTED THERAPY FOR YOUNG ADULTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKAEMIA: 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-remission allocation. Aims: To evaluate the feasibility and results of a phase I trial of intensive chemotherapy in which risk-assignment and post-remission therapy of young adults 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/m² daily on days 1, 3, and 5; i.v. etoposide 50 mg/m² daily on days 1 to 5; i.v. cytarabine 100 mg/m² 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/m² daily for 6 and i.v. cytarabine 100 mg/m² 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 without c-Kit mutations) were to receive AuSCT; high-risk pts (adverse karyotype or FLT3-ITD positive) were to receive chemotherapy; intermediate-risk pts (intermediate category (=AuSCT or ASCT); high-risk pts (adverse karyotype or FLT3-ITD-positive) were to receive ASCT.

Results: Of the 515 pts treated started and were available for the analysis. Median age was 49 (18-61) years and 52% were males. Of 429 evaluable pts, EAN cytogenetic distribution was: low-risk 11%, intermediate-risk 73% and poor-risk 15%. RUNX1/RUNX1 was detected in 49% of 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 and forty-one pts completed the consolidation phase and were risk allocated: 114 (33%) to the low-risk category (=AuSCT), 122 (36%) to the intermediate category (=AuSCT or ASCT) and 61 (19%) to the high-risk category (=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 31.4%. At the same time point of 24 months, OS and DFS in the low-risk category was 74.8% and 63.8%, respectively; in the high-risk category 42.5% and 44.8%, respectively; in the intermediate-risk category MRD negative 78.8% 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 group, using all the available sources of stem cells.

**SL12**

**GRAFT VERSUS LEUKEMIA EFFECT OF ALLOGENIC 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 analysis was performed by applying multivariable Cox regression with time-dependent covariate alloHSCT with the cumulative incidence of relapse as primary endpoint.

**Results:** MRD was positive in 120 (24%) patients after induction chemotherapy before proceeding to PRT. The latest European LeukemiaNET risk classification was similarly distributed among MRD negative and MRD positive patients. No differences were present in transplant characteristics in MRD positive and MRD negative patients. OS and RFS was significantly better in patients without MRD prior to PRT as compared to MRD positive patients (65±2% compared to 50±5% at 4 years, p<0.002, and 58±3% compared to 38±4%, p<0.001, respectively).

**Summary/Conclusions:** Overall, we conclude that our prospective results showed that MRDpos+LSCpos patients have a significantly worse cumulative incidence of relapse (HR 0.38, 95% CI 0.32-0.47) and overall survival (HR 0.36, 95% CI 0.18-0.70) as compared to MRDneg+LSCneg patients. 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 group, using all the available sources of stem cells.

**SL13**

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


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

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

**Methods:** In 242 patients who achieved morphologic complete remission, both LSC and MRD data after two cycles of chemotherapy treatment were available. MRD-positivity was defined as a percentage of MRD-positive cells above 0.1% (as compared to total amount of WBCs) and LSC-positivity was defined as a CD34+CD38-LSC percentage above 0.0000% (LSC cut-off 0.0000%; thus no CD34+CD38-LSC events measured).

**Results:** Cumulative incidence of relapse (CIR) and overall survival (OS) data were investigated for four different MRD/LSC groups: 1. MRDneg+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 a very poor prognosis. Moreover, multivariate analyses, containing all well known risk factors including risk group and post remission treatment, showed that MRDpos+LSCpos-patients have a significantly worse cumulative incidence of relapse (hazard ratio [HR] 5.89, 95% CI 3.32-10.47) and overall survival (HR 3.62, 95% CI 1.86-7.04) as compared to MRDneg+LSCneg-patients.

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

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

S114

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


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

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

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

Results: MFC-MRD results after either induction course are available for 1555 patients randomised from 4/09-12/14 (median age 51, range 0-73). Cycle 1 (C1) response data with MFC-MRD was available for 1,400 patients. 70% achieved morphological CR at this time-point; 14% had resistant disease (RD) and 16% were in partial remission (PR) according to clinician. Of patients in CR (n=984) 56% had detectable MFC-MRD (MRD+). Excluding poor-risk patients 14% of patients did not achieve CR (7% RD, 7% PR), 51% of patients in CR were MRD+. 5 year OS for MRD- vs MRD+ was 63% vs 44% vs 37% vs 25% for all patients; 69% vs 51% vs 50% vs 30% excluding poor-risk patients and 66% vs 49% vs 49% vs 30% for standard risk alone (Figure 1). The similar OS in this group between CR MRD+ and PR at C1 was maintained in NPM1wt standard risk patients and if censored at stem cell transplant. 771 patients were in CR post cycle 2 (C2) and provided MFC-MRD data. As expected, there were significant differences in 5 year OS between CR MRD- vs 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 NPM1wt standard risk patients with 5 year OS of 32% vs 64% (P=0.002) for MRD+ vs MRD-. In stratified analyses, there was some evidence that the effect of MRD positivity on OS was lower in poor-risk patients (test for trend p=0.02 for both C1 and C2). The effect of MFC-MRD status on relapse and OS appeared greater at C2 (relapse, OR 2.00(1.56-2.55), p<0.001; survival, OR 1.80(1.42-2.28) p<0.001) than C1 (relapse, OR 1.69(1.37-2.07), p<0.001; survival, OR 1.46(1.19-1.79) p<0.001). In patients with data for both time points, C2 MRD remained significant on OS when adjusting for C1 response. 24 patients converted from C1 MRD- to C2 MRD+, with a poor prognosis (15 relapses, 13 deaths). C1 MRD-/C2 MRD- had the best prognosis.

Figure 1.

Summary/Conclusions: MFC-MRD in CR post cycle 1 has similar outcomes to partial remission in younger patients with AML, particularly in patients with good and standard risk disease. Assessment of MFC-MRD post cycle 2 appears to provide additional discrimination to cycle 1: MFC-MRD in courses 1-2 may be useful in further stratifying standard risk patients.
New insights into chronic lymphocytic leukemia biology

**S115**

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

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**Background:** Recent studies have revealed the presence and prognostic impact of small mutated subclones in chronic lymphocytic leukemia (CLL) (Rossi et al 2014, Nadeu et al 2016, Rasi et al 2016). Although these studies focused only on a small subset of 5 genes, their results opened a new perspective where the proportion of cells carrying each specific driver mutation may 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-gradient pattern: the poor prognostic impact was a continuous variable directly related to the size of the subclone, and iii) CCF-clonal pattern: only mutations with a CCF above a certain threshold impacted the outcome of the patients. Combining mutations and driver CNAs, we observed at least one driver alteration in 66% of the patients. The subclonal complexity (accumulation of 1 to ≥4 driver alterations), but not the presence of subclonal driver populations, gradually shortened the time to first treatment independently of the IGHV mutational status and Binet stage. Conversely, the subclonal complexity, defined as the accumulation of driver alterations with the presence of at least one driver subclone, predicted for a worse overall survival independently of the IGHV and Binet stage. Patients with a pure clonal population (presence of one or more driver alterations in all tumor cells) had a similar overall survival than patients without any alteration.

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

**S116**

**FBXW7 MUTATIONS LEAD TO ACCUMULATION OF NOTCH1, HIF1-ALPHA AND C-MYC IN CLL CELLS**

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**Background:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with recurrent mutations that are of pathogenic and prognostic relevance. Mutations in FBXW7 are among the most common mutations in CLL, yet their functional consequences are unknown. FBXW7 is an E3 ubiquitin ligase that ubiquitylates oncoproteins like NOTCH1, HIF-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 chemotherapies 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. Here: FBXW7 mutations were analyzed via amplicon-based targeted 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 with a high mutational burden 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, HIF1-α 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 homozgyously truncated mutant cell lines in comparison to the wildtype HG3 cell line. To confirm this finding, protein levels of 5 CLL patients with FBXW7 mutations were analyzed with a similar outcome.

**Summary/Conclusions:** Mutations in FBXW7 are frequently found in CLL, especially missense and nonsense mutations affecting the WD40 domain. We hypothesize that this has functional consequences on FBXW7 substrate binding and hence results to accumulation of focal oncogenes. Thus, 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.

**S117**

**INTEGRATIVE ANALYSIS OF THE GENOME, EPIGENOME, TRANSCRIPTOME AND THREE-DIMENSIONAL CHROMATIN STRUCTURE IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background:** Different omics studies have focused on the analysis of individual layers of information in chronic lymphocytic leukemia (CLL), such as the genome, and its transcriptional regulation as well as DNA methylation, but have missed insights into other layers of the epigenome, like histone modifications, remain relatively unexplored and an integrative molecular portrait of CLL is not available yet.

**Aims:** The aim of this study was to extensively map and analyse the epigenome

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

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

Results: CLL is distinct from normal B cells for all layers of the reference epigenome of 17 CLLs and the active chromatin landscape (100 CLLs). CLL though 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 furthermore distinguish 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 promotors (H3K4me3 and H3K27ac) or active enhancers (H3K27ac and H3K4me1). More specifically, we detected 534 genomic regions with de novo gain (n=498) or loss (n=36) of active regulatory regions in CLL. Large regions (>10kb) showing de novo gain of regulatory elements in CLL (n=51), were located into, close to, or interacted in 3D space with genes important for CLL pathogenesis, e.g., LEF1, BCL2 and FMOD. Interestingly, non-coding somatic mutations in IGVH mutated CLLs accumulate in these and other active regulatory regions, likely off-target effects of the somatic hypermutation machinery. Besides changes in regulatory elements, we observed that CLLs lose poised promoters, which are replaced by repressive regions (BAFF-mediated BCR activation did not correlate with IGVH mutational status). Like IgM, BAFF induced CLL cell chemotaxis. SYK inhibition effectively antagonized survival and chemotaxis of BAFF-stimulated CLL cells. By contrast, targeting BTK or PI3K was less effective. All BCRi’s fully blocked canonical NFκB activation in BAFF-stimulated CLL cells (suggesting its dependence on BCR signaling), but none inhibited the non-canonical pathway. By contrast, pemovendistat, an inhibitor of Neddfabinating enzyme which we previously showed to abrogate TNFR-mediated NFκB activation, blocked both canonical and non-canonical NFκB activity in BAFF-stimulated CLL cells. SYK inhibitor entospleminib, but not other BCRi’s, decreased Mcl-1 expression in CLL cells co-cultured with BAFF-expressing stroma and abrogated BAFF-mediated upregulation of 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.

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**THERAPEUTIC DISRUPTION OF THE BAFF-BCR 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 employed inhibitors of Bruton tyrosine kinase (BTK, ibrutinib), phosphoinositiode-3 kinase (PI3K, idelalisib) and spleen tyrosine kinase (SYK, entospleminib). We quantified CLL cell apoptosis, migration, NFκB activity, protein and mRNA expression by flow cytometry, immunoblotting, ELISA, RT-PCR and immunocytochemistry.

Results: CLL cells co-cultured with BAFF-expressing stroma were resistant to spontaneous apoptosis (12.3±3.2% after 24 h, vs 34.8±6.2% off stroma) and chemotherapy agents (bendamustine, fludarabine). Gene expression profiling exposed the NFκB pathway gene targets as the most significantly upregulated upon BAFF stimulation (p<0.0001). We and others have shown that BAFF-expressing stroma induces canonical and non-canonical NFκB in CLL. By contrast, while BAFF led to strong activation of the non-canonical NFκB with processing of p100 (to p52) by 4 h and a 5-fold increase in p52 DNA-binding activity by 24 h, canonical NFκB (RelA) activation was less pronounced. BAFF predominantly induced Mcl-1, compared to CD40L which strongly upregulated Bcl-X. BCR is a major driver of non-canonical NFκB signaling in CLL. Thus, we studied whether BAFF co-opted BCR signaling in CLL. BAFF induced rapid (15 min) phosphorylation of the proximal BCR kinases SYKand LYN, sustained for up to 4 h, as well as ERK, in CLL cells. AKT acti-
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LOW MYBL2 EXPRESSION OBSERVED IN MYELODYSPLASTIC SYNDROME PATIENTS WITH WORSE PROGNOSIS IS ASSOCIATED WITH ALTERED DNA REPAIR MECHANISMS IN HAEMATOPOETIC STEM CELLS

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

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

Methods: In this study we used our mouse model in which animals express ~50% normal levels of MYBL2 (Mybl2+/∆∆). We characterized the ability of HSCs from young (7 weeks) and old (70 weeks) animals to respond to in vivo ionising radiation using the double strand break repair assay to measure repair of DNA replication fork instability 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 development. 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 ionizing 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 publicly available RNA-seq databases from Cancer Genomic Atlas we have identified a clear association between low MYBL2 levels and low expression of DNA-repair genes in patients with worse prognosis.

Summary/Conclusions: In summary, we have shown that decreased expression of MYBL2 leads to an imbalance in the DSB DNA-repair pathway choice, ultimately resulting in increased genomic instability of the blood cell progeny. These findings are supported by a signature of deregulated DNA-repair genes which strongly associates with low MYBL2 levels in MDS patient samples, providing a mechanistic understanding for the progression of blood disorders occurring during ageing. This study demonstrates a novel role for MYBL2 in DNA repair in HSCs and suggests that low levels of MYBL2 in human MDS could contribute to the emergence of further genetic abnormalities by deregulation of DNA-repair pathways.
The value of NGS panel sequencing to molecularly define myeloid malignancies and clarify borderline cases: a study on 39 genes in 1143 patients

Aims: To identify the deregulated pathways and gene ontology (GO) categories associated with aberrantly spliced genes in CD34+ cells and in differentiated cells of MDS-affected lineages isolated from bone marrow of patients with myeloid disorders. We analyzed the splicing characteristics, and the major cell lineages affected in MDS-affected lineages isolated from the bone marrow of each of 7 SF3B1 mutant cases. An analysis of upstream transcriptional regulators showed a significant overlap between the aberrantly spliced genes associated with each mutant splicing factor gene (in the comparison to both wildtype cases and to healthy controls) and genes regulated by several transcription factors, including EZF1. RNA-Seq also was performed on CD34+ cells, which were isolated from patients with MDS-affected lineages and from healthy controls (18 of 30 in the comparison to wildtype cases) isolated from the bone marrow of each of 7 SF3B1 mutant cases, 7 wildtype cases and 5 healthy controls, in order to explore similarities/differences between aberrantly spliced genes 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 four cell populations. GO analysis of the aberrantly spliced genes identified that 6 of the top 30 most significant categories (including 'RNA binding' and 'translation') in the comparison to wildtype cases were enriched among cases with ring sideroblasts (p<0.001). Overall, TET2 showed the highest mutation rate (25%) and was also the most commonly mutated gene in cases with "possible" MDS (36/190; 19%), reactive morphology (17/201; 8%) or even unclear morphology (19/116; 17%). Of these three subsets, five patients had only the TET2 mutation with <10% burden, which is observed in clonal hematopoiesis of indeterminate potential (CHIP), too. However, using panel sequencing in cases with possible MDS, unclear or reactive morphology revealed at least one molecular marker for clonal disease in 47% (91/199), 36% (43/118) or 17% (36/211) of cases, respectively (excluding sole ASXL1, DNMT3A, TET2 mutations with <10% burden).

Summary/Conclusions: WHO 2016 requires information on numerous genes for diagnosis, prognosis and therapeutic decisions. This challenges convention-based classification, which has been defined in all at least one molecular marker for clonal disease. Our study has identified aberrantly spliced genes and deregulated pathways associated with spliceosome 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.
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 83 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 107 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 < 0.01) and multivariate analysis (HR 6.8 [95% CI, 1.5–32], P=0.015). High frequency of leukemic transformation in the Class-II subgroup (38%) contrasted to no leukemic transformation in the Class-I subgroup. The prognostic signficance 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. Multivariat analysis also demonstrated that the predicted Class-II subgroup was independently associated with leukemic transformation (HR 7.3 [95% CI, 1.3–41], P=0.024). Finally, we compared the prognostic value of our model with that of the LSC17 score, which has recently been proposed to predict a subset of poor prognosis patients. Our model outperformed the LSC17 score in preclinical samples of leukemic stem cell signature. Our model outperformed the LSC17 score in predicting clinical outcomes of myelodysplasia, especially leukemia progression.

The Class-II signature was shown to be more dramatically up-regulated during clonal evolution of myelodysplasia than the LSC17 score, which is characterized by frequent copy number gains/amplifications in PD-L2 and PD-L1, and the close association between these genetic alterations and the therapeutic response to these agents. Recently, we have reported frequent structural variations (SVs) in adult T-cell leukemia/lymphoma (ATL) caused by human T-cell leukemia virus type-1 (HTLV-1). These SVs invariably affect 3′-untranslated region (UTR) of PD-L1, leading to promi-

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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 high-potential promising therapy for cancer, frequently showing dramatic anti-tumor responses in a wide variety of tumor types. Particularly, an exceptional response to anti-PD-1 antibodies has been demonstrated for classical Hodgkin lymphoma (HL), which is characterized by frequent copy number gains/amplifications in PD-L2 and PD-L1, and the 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 prominent PD-L1/PD-L2 expression in HTLV-1-infected cells. Additionally, tumors, leukemic cells, and potentially mixed cells of a leukemic transformation 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 < 0.01) and multivariate analysis (HR 6.8 [95% CI, 1.5–32], P=0.015). High frequency of leukemic transformation in the Class-II subgroup (38%) contrasted to no leukemic transformation in the Class-I subgroup. The prognostic significance of our classification was validated in an independent cohort of 183 patients. 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. Multivariat analysis also demonstrated that the predicted Class-II subgroup was independently associated with leukemic transformation (HR 7.3 [95% CI, 1.3–41], P=0.024). Finally, we compared the prognostic value of our model with that of the LSC17 score, which has recently been proposed to predict a subset of poor prognosis patients. Our model outperformed the LSC17 score in predicting clinical outcomes of myelodysplasia, especially leukemia progression. The Class-II signature was shown to be more dramatically up-regulated during clonal evolution of myelodysplasia than the LSC17 score, which might be the basis of a better prediction of leukemia progression in our model.

Summary/Conclusions: Comprehensive transcriptomic analysis identified two subgroups of myelodysplasia with biological and clinical relevance, which could improve risk prediction and treatment stratification of myelodysplasia.
shown previously to increase FOXO1 nuclear localization and activity, have been linked to diminished survival in DLBCL patients uniformly treated with rituximab-based immunotherapy. Although the contribution of FOXO1 mutations to the therapeutic resistance of B-NHLs becomes apparent, the molecular mechanism underlying this phenomenon has not been explained so far. The diminished levels of CD20 on the cell surface of tumor cells are among several potential mechanisms underlying the resistance to treatment with anti-CD20 monoclonal antibodies.

Aims: We have recently reported that the tonic BCR signaling activates FOXO1, and that inhibitors of the downstream BCR signaling pathways down-regulate CD20 expression. Therefore, here we sought to determine whether FOXO1 might regulate the abundance of CD20 on the surface of tumor cells thus influencing the response to rituximab-based therapies.

Methods: We used CRISPR/Cas9 genome editing technology and lentiviral transduction to study the role of FOXO1 protein in CD20 regulation. qRT-PCR and Dual Luciferase Assays was done to determine the influence of FOXO1 on CD20 transcription. To explore if tumor suppressor function of FOXO1 was retained in mature B cells, we performed FACS 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 in accordance with the guidelines and were approved by the Ethics Committee. Results: To determine the potential role of FOXO1 in CD20 regulation, we disrupted FOXO1 focus using the CRISPR/Cas9 genome editing technology in Raji cells. In vitro complement-dependent cytotoxicity assay we show that ablation of FOXO1 results in upregulation of CD20 levels and improved rate of rituximab efficacy. To see whether FOXO1-dependent up-regulation of CD20 transcription influences tumor suppressor function we performed in vivo experiments where we 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 B cell lymphoma cell lines, we show that PI3K-AKT inhibition of FOXO1 activity upregulated surface CD20 levels. Moreover, we demonstrated that FOXO1 regulates the CD20 promoter activity. In different B-cell lymphoma cell lines MK-2206 and GDC-0068 significantly downregulated the levels of MS4A4A transcript (encoding CD20). Finally, using both EMSA and ChIP assays we detected specific binding of FOXO1 to the MS4A4A 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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Investigations of the NIPA knockout mouse in a clinical relevant ALCL model

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

Methods:

Primary Nipa-/-MEFs infected with NPM-ALK were plated in soft agar 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 immunphenotype and clinical presentation.

Results:

Primary Nipa-/-MEFs infected with NPM-ALK showed significantly reduced colony formation potential upon NPM-ALK expression (38 vs 79 CFUs; p<0.001). These results were substantiated in human and mouse cell lines, where significantly reduced proliferation ability was observed in NIPA downregulated NPM-ALK-expressing Ba/F3 cells (74% of wt; p<0.01) 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-CreTg(2)Nipaflx/floxMSNAIE infected bone marrow cells showed significantly prolonged survival (14.9 vs 12 days in wt). Morphologically, mice presented with enlarged thymi, splenomegaly, lymphadenopathy, and bone marrow infiltration. Immunophenotyping showed a pure T-cell phenotype in Nipa-/- lymphomas, thus resembling wildtype. In a long-latency model of NPM-ALK expression in enriched HSCs, a significantly prolonged survival (110 vs 80 days; p<0.01) and reduction of spleen colonies (10 vs 28 colonies/spleen; p<0.001) in mice transplanted with MigNPM-ALK:Nipa-/- 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.

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eTHERAPY-BTHALIAL 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 lenogastim and plerixafor as source of HSCs and a conditioning regimen based on myeloablative treosulfan and thiotepa favoring efficient engraftment of corrected cells with reduced toxicity (TIGET-BTHAL; EudraCT number 2014-004860-39).

Methods: On the basis of extensive efficacy and safety preclinical studies, the clinical trial TIGET-BTHAL was approved and started in 2015 at Scientific Institute San Raffaele, Milano, 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-thal/β-thal) have been treated with GLOBE-transduced CD34+ cells at a dose of 16x10⁶-19.5x10⁶ 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.

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LUSPATERCEPT INCREASES HEMOGLOBIN AND DECREASES TRANSFUSION BURDEN IN ADULTS WITH B-TALASSEMIA


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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, 2014) and increased Hgb levels in patients with ineffective erythropoiesis treated 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
S130
DENOSUMAB INCREASES BONE MINERAL DENSITY IN PATIENTS WITH THALASSEMAIA MAJOR AND OSTEOPOROSIS: RESULTS OF A RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE BLIND, PHASE 2 CLINICAL TRIAL


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Background: Thalassemia major (TM) is a genetic disorder caused by an inherited defect of the β-globin chain, which results in a decreased production of hemoglobin. It is a common disorder with an estimated prevalence of 1 in 100-200 live births in the Mediterranean and Southeast Asian regions. Thalassemia major is characterized by severe anemia, iron overload, and complications related to bone disease.

Methods: This French retrospective study included patients who successfully received allogeneic HSCT between 1985-2012 and were alive at least 2 years after HSCT. Study results were explored in 107/134 patients (79%) who performed HSCT for beta-thalassemia. The patients were comprised of 58 males and 49 females. The median age at HSCT was 5.9 years (8 month-26 years).

Results: Of the 134 patients, 97% achieved at least 10% bone marrow engraftment (bweight SDS, SF, Hb values). In 95% of cases, the donor was a matched sibling or an unrelated donor. The median number of days to neutrophil engraftment (NCAT02604433).

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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 (cRBC), such as iPSCs would allow selection of ‘universal donor’ RBC, or provide an autologous end product with the absence of immune reactions.

Results: The in vitro production of RBC has proven to be successful, however there are barriers to overcome prior to clinical application. (e.g. xeno-free culturing methods, scale up cultures to obtain transfusion units (1-2*10^12 RBC), such as iPSCs would allow selection of ‘universal donor’ RBC, or provide an autologous end product with the absence of immune reactions.

Summary/Conclusions: Such an unlimited production of cRBCs would be essential to allow directed colony differentiation using a feeder-free monolayer approach.

S133
FUNCTIONAL PROTEOMICS IDENTIFIES SETD2 AS A CRITICAL EFFECTOR OF MLL FUSION PROTEINS TO SAFE-GUARD GENOMIC INTEGRITY
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Background: Acute Myeloid Leukemia (AML) frequently harbors chromosomal rearrangements involving the Mixed Lineage Leukemia (MLL) gene. More than 65 different MLL fusion genes exist and many of them have been described to act as strong cancer drivers. While critical effectors of different MLL fusion proteins that are presumed to employ different mechanisms of oncogenic transformation.

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

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

Results: Characterization of the protein complexes nucleated by 7 MLL fusion proteins by affinity purification coupled to mass spectrometry (AP-MS) revealed a densely interconnected protein-protein interaction network of 963 proteins, comprising previously known MLL-interacting protein complexes (such as PR2C 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.

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 mechanistic 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 mutant MLL 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 Cebpα+/−AML mouse model. Cellular competition assays were used to assess changes in proliferative capacity and apoptosis of MLL−/−AML cells. Further, MLL−/−AML activity was inhibited by the small molecules that block the Menin-MLL interaction. In both cases, proliferative, 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 p30 isoform and the MLL (xenotransgenic) promoter, indicating functional cooperativity in gene regulation. To investigate the importance of different, annotated functional domains within the MLL protein in the context of C/EBPα p30 expression, we introduced targeted mutations across the MLL gene in Cebpα+/−AML cells using the CRISPR/Cas9 system. This analysis revealed a strong dependence of Cebpα+/−AML on the expression of an intact MLL protein. Surprisingly, loss of the enzymatic activity of MLL by mutagenic targeting of the SET domain did not significantly affect cell survival. In contrast, cells were particularly sensitive to mutagenesis of the Menin-binding motif in MLL. MLL targeting strongly induced myeloid differentiation in Cebpα+/−AML cells as measured by increased levels of CD11b and CD11c markers. To identify potential functional targets, we performed upon pharmacological perturbation of the MLL/SET complex, we used miR-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 Cebpα+/−AML cells. RNA-seq analysis of Cebpα+/−AML cells after treatment indicated dysregulation of gene expression 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 Cebpα+/−AML cells were 2.6-fold more sensitive than other leukemia cell lines of mouse and human origin.

**Summary/Conclusions:** We found that inhibition of C/EBPα p30 by CRISPR/Cas9-mediated mutagenesis results in clinical and metabolic improvements in vivo. In conclusion, targeting PU.1 provides a promising strategy for the treatment of acute myeloid leukemia.

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**S135**

**FUNCTIONAL AND TRANSCRIPTOMIC IMPACT OF INHIBITING 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 PU.1, which is a major transcriptional target of PU.1, is required for PU.1-induced transcriptional changes that contribute to leukemogenesis. Therefore, we aimed to investigate the sensitivity of CEBPα mutant PU.1 to perturbation of PU.1 function.

**Methods:** We used two alternative approaches: RNA interference and newly developed pu.1 reporter assays. We found that inhibition of PU.1 with different shRNAs led to a significant decrease in cell growth and colony forming capacity, increased apoptosis, and disrupted serial replating capacity of PU.1+/−AML cells, and a majority of primary AML cell samples. ChIP and expression analysis showed that the compounds disrupt PU.1-1promoter interaction and lead to downregulation of canonical PU.1 transcriptional targets in AML cells, confirming on-target activity.

**Results:** 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.
FLT3 TK activity may improve the eradication of FLT3mutAML cells. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK activity may improve the eradication of FLT3mutAML cells.

Summary/Conclusions: Our data suggest that upon AC220 treatment, glutamine metabolism becomes a critical metabolic dependency in FLT3mutAML. Glutamine metabolism is mostly channelled towards glutathione production, while also supporting the CAC and both these fates contribute to its protective effects following FLT3 TK inhibition by respectively counteracting oxidative damage and sustaining macromolecule biosynthesis and cellular energetics. These data predict that a combined inhibition of glutamine metabolism and FLT3 TK activity may improve the eradication of FLT3mutAML cells.

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 engraftment potential (rEC-MPPs). Adult, non-lymphatic ECs isolated from various organs of Runx1-IRES-GFP reporter mice were transduced with FGRS and co-cultured in direct contact with vascular niche.

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

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

S138
MARROW 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 (IR) etal. However, it is still unclear if bone marrow mesenchymal stem cells (BMSCs) can also form TNT to communicate and rescue injured bone marrow derived endothelial cells (BMdEC) and promote it recovery from chemotherapy stress. In our study, we set out to test the hypothesis that BMSCs can rescue suffering endothelial cells by transferring mitochondria to endothelial cells through nanotubes.

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

Methods: We established two direct co-culture system for human primary bone marrow mesenchymal stem cells (BMSCs) and bone marrow derived endothelial cells (BMdECs) respectively.

Results: Firstly, We observed the TNTs formed between BMSCs and endothelial cells including the TNT structure between BMSCs and HUVECs or BMdECs are composed with F-actin, microtubule in addition to membrane. Live cell imaging showed the two xenogeneic cells form TNTs by retaining a thin thread of membrane upon dislodge.
ment. Besides, we observed that TNT formation may alter bone marrow microvascular stem cell and endothelial cell cytoskeleton (Ara-C) treatment. Single-cell analysis showed that stressed endothelial cells and cell lines in the early stages of apoptosis caused by cytarabine (Ara-C) treatment form TNT to interact with untreated BM/MSCs and then mesenchymal stem cells transport mitochondria to injured endothelial cell or cell line in vitro. The TNT formation was inhibited by incubating with an F-actin-depolymerizing drug and tubulin-depolymerizing drug, indicated that these TNTs transferring mitochondria have a distinct cytoskeletal composition which composed with F-actin and microtubule.

Our results also suggest that the delivery of functional mitochondria from endothelial cells to BM/MSCs can mediate the recovery of injured BMUVECs from the apoptosis, contribute to proliferation and remodel the formation of capillary-like structures in Matrigel-coated plates of HUVECs suffer from chemotherapy stress of Ara-C.

Summary/Conclusions: BM/MSCs can transfer mitochondria via TNTs formed between BMUVECs and rescued endothelial cells under stress, which can alleviate apoptosis of stressed endothelial cells, relieve its proliferation inhibition and alter its formation of capillary-like structures. Our study offers the clues to help know about cell-cell communication of niche components in the HSC niche in bone marrow.

S139 SHORT-TERM FEEDING OF A HIGH-FAT DIET DISTURBS LIPID RAFT/TGF-BETA SIGNALING-MEDIATED QUIESCENCE OF HEMATOPOIETIC STEM CELLS IN C57BL/6J MOUSE BONE MARROW F. Hermetet1,2, 2. J.-P. Pais de Barros2,3, 2. L. Delva1,2, 2. R. Quéré1,2 1Signaling and Physiology in Hematological Research, UMR1231 Inserm / Université Franche-Comté / AgroSup, Dijon, France 2Institute of Immunohematology, INSERM/CEA/Universite Paris 7/Universite Paris 11, 7UMR1163, Paris Descartes–Sorbonne Paris Cité University, Imagine Institute, , Paris, France 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 inflammation 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. Infarction 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% fat) or an HFD (62% fat), over a short period of 4 weeks, to investigate the direct impact of such a diet on hematopoiesis.

Results: While fat intake led to an increase in plasma cholesterol levels, mice did not develop obesity, and no inflammatory monocytes and no modulation of pro- and anti-inflammatory cytokine levels were detected in blood and BM, respectively. The impact was 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.

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**Gene therapy, immunotherapy and vaccination 1**

**S141**

**WILMS’ TUMOR 1 RNA-ELECTROPORATED DENDRITIC CELL VACCINATION AS POST-REMISSION TREATMENT TO PREVENT OR DELAY REPOPULATION 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 polychemotherapy, but at very high risk of relapse with autologous DCs loaded with the peptide-encoding Wt1 (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 following the 4th dose of vaccination were stained with a tetrameric HLA-A*0201 WT1 peptide-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, human IL-7, and IL-15 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 complete remission by DC vaccination only. In the remaining 4 responding patients, the clinical response was characterized by stable disease as demonstrated by elevated but stable WT1 transcript levels in blood for 3-12 months and stable blood values without blasts. Five-year overall survival was 40%, as compared to 24.7% in the SEER data of the National Cancer Institute; it was significantly higher in responders than in non-responders (53.8% vs 25.0%; P<0.001). 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, including 5 who relapsed after DC vaccination; 2 proceeded to allogeneic stem cell transplantation, while the 3 other patients were brought back into CR by chemotherapy alone, 2 of them surviving more than 7 and 4 years respectively after achieving CR. Long-term clinical response was correlated with increased circulating frequencies of poly-epitope WT1-specific tetramer+CD8+T-cells. Long-term overall survival was correlated with interferon-γ and tumor necrosis factor-α WT1-specific responses in DTH-infiltrating CD8+T-lymphocytes.

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

**S142**

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


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**Background:** To test the safety and efficacy of the CAR T cell modality in relapsed/refractory multiple myeloma (MM), we have designed a second-generation CAR construct targeting B cell maturation antigen (BCMA) to redirect T cells to MM. bb2121 consists of autologous T cells transduced with a lentiviral vector encoding a novel CAR incorporating an anti-BCMA scFv, a 4-1BB costimulatory motif and a CD95-zeta T cell activation domain. We will report updated safety and efficacy results following promising initial results (Berdeja et al. ENA 2016).

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

**Methods:** CRB-401 (NCT02659892) 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. The primary endpoint is to determine if ≥50% BCMA-expressing 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 an infusion of bb2121. The study follows a standard 3+3 design with planned dose levels of 5.0, 15.0, 45.0, 80.0 and 120 x 10⁷CAR+T cells.

**Results:** As of November 18, 2016, 11 patients had been infused with bb2121 in the first 4 dose cohorts, and 9 patients had reached at least 1 month of follow-up. As of data cut-off, no dose limiting toxicities, and no >Grade 2 neurotoxicities or cytokine release syndrome (CRS) had been observed. Grade 1-2 CRS has been reported in 8/11 (73%) treated patients. All patients treated with doses >5.0 x 10⁷ CAR+T cells (≥45.0 x 10⁷) have had grade 3-4 CRS. High dose 45.0 x 10⁷ CAR+T cells in 1 patient led to grade 5 neurotoxicity and adverse impact survival.

**Summary/Conclusions:** bb2121 demonstrates promising results levels above 5.0 x 10⁷ CAR+T cells, 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.
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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 did not achieve 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 infusion of packed red blood cells and platelets. Six FA patients aged 3-6 years underwent collections after mobilization of significant numbers of CD34+ cells 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 in the hematopoietic progenitor cells, deduced from the acquired resistance of the colony forming cells to mitomycin C (15% of BM CFCs survived to 10 nM MMC), have been demonstrated after 9 months of follow up in one of the patients. Summary/Conclusions: Our preliminary results show that 1) HSCs collection is both safe and effective 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.

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TARGETING FLT3 WITH CHIMERIC ANTIGEN RECEPTOR T CELLS CONFRS 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 signaling via tyrosine kinase 3 (FLT3) as a novel CAR target in acute myeloid leukemia (AML). FLT3 is a homodimeric transmembrane protein with uniform expression on AML, irrespective of cytogenetic and histomorphologic subtype. FLT3 provides survival signals to AML blasts and is a key driver of leukemia-genesis in AML cases with internal tandem duplication (FLT3-ITD). These attributes suggest FLT3 may be an ‘Achilles heel’, making AML blasts susceptible to CAR T-cell mediated recognition and elimination. Aims: We therefore explored the anti-leukemia efficacy of FLT3-CAR modified T cells against FLT3-ITD+ and FLT3 wild type AML in pre-clinical models in vitro and in vivo. Methods: A FLT3-CAR comprising a single-chain variable fragment (4G8), fused to an IgG-Fc spacer, and signaling module with CD3 zeta and CD28 was encoded in a lentiviral vector (epHIV7) for gene-transfer into CD8+ and CD4+ T cells of healthy donors (n=4) and AML patients. CAR T-cell mediated cytolytic activity was evaluated in FACS-fluorescence-based assays, cytokine production analyzed by ELISA and proliferation assessed by CFSE dye dilution. Immunodeficient NSG mice were engrafted with AML cell line (Molm-13) or primary AML blasts and treated with 5x10^6 CAR-modified or control T cells (CD8:CD4 ratio=1:1).

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

Summary/Conclusions: Collectively, our data demonstrate that T cells expressing a FLT3-specific CAR mediate potent reactivity against FLT3 wild type and FLT3-ITD+A MLL in vitro and in vivo, and establish FLT3 as a novel CAR target in AML. FLT3-ITD positivity identifies a high-risk AML subgroup that may particularly benefit from adoptive therapy with FLT3-CAR T cells, e.g. in order to achieve ‘minimal residual disease’ (MRD) negativity prior to allogeneic HSC transplantation. Our data further suggest that in contrast to CD33 and CD123, which are pursued as alternative CAR targets in AML, targeting of FLT3 may preserve a fraction of normal HSC and enable the implementation of CAR therapy outside the transplant setting.
**Background:** Allogeneic haploidentical hematopoietic stem cell transplant (HSCT) offers curative therapy for children who lack a matched HLA-identical donor with hematopoietic disorders such as Primary Immune Disorders (PID), hemoglobinopathies, erythroid disorders and acute leukemias. T-cell depletion mitigates the risk of GVHD after haplo-HSCT, but is associated with extended immunodeficiency, leading to complications due to infections. We have performed TCR-depleted haplo-HSCT with post-transplant infusion of BPX-501 gene modified T-cells to allow for more rapid immune reconstitution. Upon occurrence of GVHD, administration of rimiducid (AP1903) dimerizes the Cas9 suicide switch and rapidly induces apoptosis of the transduced BPX-501 cells and mitigates the GVHD.

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

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

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

<table>
<thead>
<tr>
<th>Non-Malignant</th>
<th>N=66</th>
<th>Malignant</th>
<th>N=38</th>
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<tbody>
<tr>
<td>SCD</td>
<td>11</td>
<td>ALL (CR1 (21 CR2)</td>
<td>24</td>
</tr>
<tr>
<td>WAS</td>
<td>6</td>
<td>AMI</td>
<td>14</td>
</tr>
<tr>
<td>CDG</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>T-cell Myeloid</td>
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<td></td>
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<tr>
<td>Sickle Cell Disease</td>
<td></td>
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</tr>
<tr>
<td>Fanconi Anemia</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIU</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>20</td>
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</tbody>
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**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, Grade 3 and 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, attributed to the allotgift, not BPX-501. Rimiducid was used in 4 patients with Grade 2 GVHD with rapid resolution of symptoms, as it did in the severe cGVHD patient. In both malignant and non-malignant patients. CD3, CD4, CD8 (Figure 2B) and B-cells (Figure 3C) immune reconstitution was brisk. CD3+/CD19+ T-cells were detectable at one year via flow cytometry analysis of peripheral blood. In Wiskott-Aldrich patients, platelet recovery remains in the normal range at 180 days with mean platelet counts of 246.3±10^3/uL. 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 dimer 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.

**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:** 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.

**Methods:** 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+ 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 in SCD and β-Thal.

**Summary/Conclusions:** 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 in SCD and β-Thal.

**Background:** 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:** 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 CRISPR/Cas9 derived from healthy-donor bone marrow (n=6). Cas9 delivery as recombinant protein improved cell viability when compared to mRNA-based delivery (88.8±3.7% compared to 75.5±3.3%, Mean±SD, n=56 for each) with no observed reduction in editing efficiencies. To investigate gene editing impact on HbF, edited cells were erythroid differentiated from healthy donors as well as SCD and β-Thal patient samples. 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+CD19+CD45RA− long-term repopulating HSPCs and bulk CD34+ HSPCs (87.9±6.4% compared to 89.7±5.9%, Mean±SD, n=4) at 2 weeks. We confirmed that editing levels of edited cells in immunocompromised mice were similar to control cells (% human CD45 in peripheral blood = 28.6±6.9% 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.
for a selected guide RNA confirmed no detectable genomic cleavage at over 50% of 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 deletions in both hematopoietic and non-hematopoietic compartments. 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 off-target editing. These experiments support the further development of specific CRISPR/Cas9 editing strategies of HSPCs to treat SCD and β-Thal patients.

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

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

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

Methods: We have developed and characterized two independent GEMMs, in addition to the Pax5+/−-infected model (1), which were exposed to a common infection environment. These represent childhood BCR-ABL-1p190 BCP-ALL and the most common subtype ETV6-RUNX1 BCP-ALL. Both model systems ensure Scg11-directed expression of BCR-ABL1p190 or ETV6-RUNX1 in HSCs and in ETV6-RUNX1 mice develop BCP-ALL at a low penetrance (10.75%; 10 out of 93) with a CD19+B220+IgM-cell surface phenotype and manifested with blast cells in the peripheral blood (PB) and clonal immature BCR rearrangement. Whole exome (WES) and whole genome sequencing (WGS) was performed of murine BCP-ALL on a HiSeq 2500 (Illumina) platform.

Results: Pax5+/− and Scg1-ETV6-RUNX1 mice develop BCP-ALL only after exposure to infections whereas the Ptx5+/− mice develop BCP-ALL independent of exposure to common infection. The molecular mechanism leading to BCP-ALL identified in the infection dependent GEMMs is determined by the genetic predisposition (Pax5+/− or ETV6-RUNX1). Pax5+/− mice acquire constitutive activating Jak3 mutations (6/9) in a susceptible B-cell precursor population (extraocular mature B cells (1)). On the other hand, Scg1-ETV6-RUNX1 mice develop BCP-ALL at a low penetration (10.75%; 10 out of 93) with a CD19+B220+IgM-cell surface phenotype and manifested with blasts cells in the peripheral blood (PB) and clonal immature BCR rearrangement. High expression of Recombination Activating Gene 1 (Rag1) and loss of function mutations in ETV6 were identified in murine BCP-ALL and are well known in the context of human ETV6-RUNX1 leukemia. Additionally we identified a high proportion of mutations in genes implicated in histone modification, i.e. Kdm5c (no. 408) causing a premature stop. CRISPR-Cas9 knock down studies of KDM5C in a precursor B-cell line revealed facilitated Rag1/2 binding to the open gene complex ((H3K4me3) as a potential molecular mechanism. Rag1 off target activity in pre-leukemic ETV6-RUNX1 HSCs/PC after exposure to infection. In contrast to Pax5+/− and Scg1-ETV6-RUNX1 mice, Sca1-BCR-ABL-1p190 mice develop BCP-ALL independent of exposure to common pathogens by reprogramming of a HSC/PC and subsequent loss of Pax5. Summary/Conclusions: In summary, exposure to common pathogens can trigger childhood BCP-ALL based on Pax5 loss of function or exposure to the common ETV6-RUNX1 rearrangement. However the underlying molecular mechanism (Jak-Stat signaling in Pax5+/− mice and histone modification in ETV6-RUNX1 mice) triggered by exposure to common infection is determined by the genetic predisposition and hand BCP-ALL sublines emerge on the basis of a potent oncogene (such as BCR-ABL1p190) can develop independent of exposure to infection. These findings are important for encouraging the prospect of novel interventions that might help to prevent or treat a significant proportion of childhood BCP-ALLs.

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

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

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

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

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

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TARGETED SINGLE CELL SEQUENCING TO IDENTIFY MUTATIONAL HIERARCHY IN T-CELL ACUTE LYMPHOMA LEUKEMIA J. de Bie1, S. Demeyer1, E. Gerders1, A. Uyttebroeck2, N. Boecx3, J. Cools4
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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, each with a distinct mutational landscape that are likely the result of clonal branched evolution. Understanding this clonal evolution and the order at which mutations are acquired can provide improved insights into the origins of leukemia relapse.

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

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

Results: We detected between 2 and 4 separate clones in each T-ALL patient sample. Every patient harboured one dominant clone comprising 46 to 98% of all single cells that was highly mutated, accompanied by a number of smaller subclones carrying fewer mutations. No mutually exclusive mutations, fusion genes or deletions were observed between the clones arguing against independent leukemic clonal initiation events. Instead, a more stepwise clonal hierarchy was defined, indicating that the accumulation of mutations can occur in progenitor cells or in committed T-cells. During remission, we could no longer detect HSPCs with all leukemic mutations.

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 or deletions.

P152
BCL2 INHIBITION AS NEW THERAPEUTIC OPPORTUNITY FOR RPL10 R98S MUTANT PEDIATRIC T-ALL
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Background: The ribosomal protein L10 (RPL10) R98S mutation occurs in 8% of pediatric T-cell acute lymphoblastic leukemia (T-ALL) cases. RPL10 R98S leads to a proliferation defect in lymphoid cells but its oncogenic contribution in pediatric T-ALL remains unclear. Treatment intensification and risk stratification has reduced the relapse rate of T-ALL to ~15% but further improvements will require strategies that focus on specific subtypes as RPL10 R98S; if the long-term sequelae of toxic therapy are to be avoided.

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

Methods: Quantitative label-free proteomics was used to screen for protein differences between RPL10 WT and R98S expressing Ba/F3 cells. Hits were confirmed by western blotting and functional analyses. RPL10 R98S knock-in mice were generated. Somatic knock-ins were confirmed by sequencing and ribbon PCR. Human pediatric T-ALL samples were transplanted into NOD-SCID/IL2γc (NSG) mice for in vitro and in vivo inhibitor studies.

Results: The RPL10 R98S mutation provided a cell survival advantage in Ba/F3 cells and in serial re-plating assays of 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, explaining the observed elevated levels of reactive oxygen species (ROS) in RPL10 R98S cells that could not be scavenged by the increased catalase expression. High ROS levels and enhanced 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 to regulation 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: Bcl-2 overexpression induced by peroxisomal hyperactivation was defined as new target in RPL10 R98S defective T-ALL. Additionally, due to peroxisomal hyperactivation, a peroxisomal oxidase involved in purine degradation may have contributed to the observed increased levels of uric acid, which was elevated above reference levels in the blood of RPL10 R98S mutant pediatric T-ALL patients at diagnosis (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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TRANSLATE ANALYSIS OF THE T-ALL ASSOCIATED RIBOSOMAL PROTEIN L10 R98S MUTATION REVEALS ALTERED SERINE METABOLISM

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Background: We previously described a recurrent arginine-to-serine mutation on residue 98 (R98S) in ribosomal protein L10 (RPL10), with a frequency of 8.6% in pediatric T-ALL cases. The R98 mutant residue contacts the catalytic core (peptidyltransferase center, PTC) of the ribosome and causes ribosome biogenesis, Ptd RNA, tRNA folding defects on A1 and tRNAs. 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, polysize RNA sequencing and ribosome footprinting showed respectively 57 and 22 genes with significantly higher translational efficiency in RPL10 R98S, and 22 and 29 genes with reduced translational efficiency. Among them, we also found genes involved in T cell differentiation and proliferation. In particular, Mapk6 presented reduced translational efficiency in the ribosome footprinting, potentially due to differences in ribosome occupancy of an upstream ORF, whereas the transcription factor Ikrf2, 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 with Casf2β/2, Jak1 and several Stats being 1-3-fold elevated at the protein level and higher translation efficiency for Lfng, Il10ra, Cish and Ikrf2. Another interesting candidate showing 5-fold upregulated protein levels was phosphoserine phosphatase (Psp), a key enzyme in serine biosynthesis. Ribosome footprinting revealed that this upregulation originates from a combination of higher transcription and translational efficiency of the encoding gene. Elevated Psp protein levels were confirmed by immunoblotting in the RPL10 R98S Ba/F3 cells and in hematopoietic cell lines derived from RPL10 R98S knock-in mice. Interestingly, harvested medium from RPL10 R98S Ba/F3 cells contained higher residual serine levels versus RPL10 WT mouse lymphoid Ba/F3 cells. Our data suggest that RPL10 R98S expressing cells enhance their endogenous serine production, leaving more serine that can support survival of neighboring cells.

Summary/Conclusions: Analysis of the translational changes associated with the RPL10 R98S mutation reveals alterations for genes involved T cell differentiation and proliferation: the atypical MAP kinase Mapk6, whose reduced translational efficiency still needs to be validated at the protein level, and the transcription factor Ikrf2. Alterations were also found in the JAK-STAT signaling, an established oncogenic cascade in T-ALL. Moreover, this is the first description of a mutation in T-ALL that is linked to alterations in cellular serine biosynthesis.

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

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

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

Methods: 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 protocol screening of MLL-rearranged ALL cell lines 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 translation 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 killing induced by these drugs.

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

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

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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.

Materials and Methods: TP53 was sequenced by the method of Sanger. Drug sensitivity was assessed by clonogenic survival of wt p53. In contrast, nucleotide analoga showed no p53 inducible N248P in MHH. Three ALL cell lines were used as controls. Topoisomerase II inhibitors upregulated TP53 expression in 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 killing induced by these drugs.

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

Aims: We identified 20 different TP53 mutations in 34 patients. We classified TP53 mutations into ‘hot spot’ (R175, G245, R248, R273 and R282), 1Children’s Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, 2ACRF Drug Discovery Centre for Childhood Cancer, Children’s Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney, New South Wales, 3Telephone Kids Institute, University of Western Australia, Perth, Western Australia, Australia.
tion, IC50 measurements showed that TP53 mutations lead to resistance against topoisomerase II inhibitors and alkylating agents, but not against other drugs. The upstream pathway of p53 (CHK1, CHK2) and DNA damage recognition (γH2AX) were not impaired in the six ALL cell lines. To study the effect of TP53 mutation on resistance to treatment in more detail, we focused on the R248P mutation, located in hot spot codon 248, that we found in a relapsed patient with non-response to treatment and in the MHH cell line. Using a CRISPR/Cas9 knockut (KO) of endogenous p53 and lentiviral based re-expression in NALM-6, we generated p53 KO, and KO+wt p53, KO+R248P and KO+GFP cell lines. The KO cells showed a similar resistance to DNA damage inducing drugs as KO+R248P cells. Overexpression of wt p53 in KO cells restored sensitivity to DNA damaging drugs. In contrast to wt p53, R248p did not inhibit cell proliferation under drug treatment. We found that this mutant was unable to induce downstream targets of p53 (p21, BAX). Moreover, ChiP-seq showed that R248p cannot bind the promoter and induce expression of typical p53 targets MDM2, p21, BAX, BCC3/PUMA, FA1 and PUMA. This result indicates that R248P is defined as a frameshift and the consensus element of p53. However, the binding motif analysis showed that the R248P mutant still binds DNA at a different and purine-rich sequence. In summary, R248p leads to wt p53 function and mediates resistance to topoisomerase II inhibitors and alkylating agents.

Summary/Conclusions: Overall, our results show that mutations affecting TP53 hot spots, in particular codon 248, are associated with resistance of ALL cells to chemotherapy and reveal first insights into underlying mechanisms and pathways.

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GENETIC ACTIVATION AND THERAPEUTIC TARGETING OF PIM1 IN T-CELL ACUTE LYMPHOBластIC LYMPHOMA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) and T-cell acute lymphoblastic lymphoma (T-LBL) are aggressive immature T-cell malignancies that are considered one disease entity according to the World Health Organization (WHO). Both T-ALL and T-LBL are often characterized by improper T-cell receptor (TCR) re-combinations leading to aberrant activation of proto-oncogenes. PIM1 is a constitutively activated kinase that is involved in cell cycle, translation and apoptosis. Finally, we evaluated the therapeutic potential of PIM1 inhibition.

Aims: Despite some genetic and phenotypic similarities between T-ALL and T-LBL, T-ALL risk group stratification cannot be extrapolated to T-LBL patients. Aims:

Methods: We used an IL-7-dependent leukemia T-cell line (TAIL7) and “primary” cells from patient-derived xenografts (PDX). We used inhibitors of PI3K (LY294002), mTOR (rapamycin), MEK1/2 (U0126) and ULK1 (MRT68921). Analysis of viability and cell size was performed by flow cytometry. Signaling pathway activation and LC3-II-III conversion was performed by western blot analysis. LC3 puncta formation was assessed by confocal microscopy.

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

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

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

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Summary/Conclusions: All together, our study identifies PIM1 as a putative oncogene in T-LLB and suggests that inhibition of this serine/threonine kinase could serve as a novel therapeutic strategy in this aggressive T-cell neoplasm.
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**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 >80% 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/LKT3JAK 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 infants and high-risk childhood B-ALL and can be therapeutically targeted in vivo with the selective SYK inhibitor entospletinib.

**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 oral administration of ento and control animals at both 0.03% and 0.07% chow formulations (representative data in Figure 1; p<0.05). Steady state concentrations were maintained during the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively). PD studies demonstrated dose-dependent in vivo inhibition of pERK measured in human leukemia cells within spleens of ento-treated mice without alterations in total SYK protein levels. In general, PD inhibition of SYK target phosphoproteins was more pronounced in 0.07% ento-treated animals.

**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-MLL3 (ALL3103) and Ph-like NUP214-ABL1 (NH011) PDX models significantly inhibited ALL proliferation in vivo versus control animals at both 0.03% and 0.07% chow formulations (representative data in Figure 1; p<0.05). Steady state concentrations were maintained throughout the study duration with terminal PK values of 3.3 (± 0.5) and 7.9 (± 1.0) μM (0.03% and 0.07% ento arms, respectively). PD studies demonstrated dose-dependent in vivo inhibition of pERK measured in human leukemia cells within spleens of ento-treated mice without alterations in total SYK protein levels. In general, PD inhibition of SYK target phosphoproteins was more pronounced in 0.07% ento-treated animals.

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

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

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

**Aims:** Given the importance of NOTCH signalling in human cancers, several therapeutic approaches have been utilized to block NOTCH signalling. Two of these strategies are: a) the use of monoclonal blocking antibodies (mAbs) against NOTCH ligands and receptors and b) the use of small molecule gamma-secretase inhibitors (GSIs). However, these approaches can only be effective if tumor cells express full-length ligand or receptor molecules. As validation of NOTCH as a therapeutic target, clinical activity of these in clinical studies were observed in various trials for some of these inhibitors (mAbs, GSIs), but treatment and exposure were usually limited due to toxicities, mainly related to gastro-intestinal adverse events. On the contrary, in human cancers harbouring NOTCH gene fusion due to chromosomal translocations or specific NOTCH mutations, the use of mAbs and GSIs will have very limited clinical benefits. Cellestia has decided to follow a disruptive approach, by blocking NOTCH signalling in the most downstream part of the NOTCH cascade, at the level of the NOTCH transcriptional activation complex, using small molecule inhibitors.

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

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

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

IKZF1Δ4-7 can be easily screened by PCR but does not predict outcome in adults with acute lymphoblastic leukemia; 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 leukemia (ALL) and have been reported as independent prognostic factors for poor outcome. IKZF1Δ4-7, resulting in the dominant negative Ik6 isoform is the most common single IKZF1 deletion.

Aims: We aimed to generate and validate a simple, PCR-based screening assay for 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 IKZF1-expressing cell line SUP-B15 and calculated to be 0.001%. A total of 95 samples were also tested using the MLPA P335 kit to detect the full spectrum of IKZF1 deletion. Sanger sequencing confirmed the breakpoints in 27 cases.

Results: The median age of the patients tested was 46 years (range 25-65). Overall IKZF1Δ4-7 was detected in 97/490 (20%) patients but the frequency varied by genetic subtype. Patients with BCR-ABL1 fusion had the highest IKZF1Δ4-7 frequency (46/150, 31%) followed by patients with B-other ALL (29/154, 19%). Patients with other classic cytogenetic abnormalities harboured significantly fewer IKZF1Δ4-7 – low hypodiploidy (3/26), MLL gene fusions (3/31), t(1;19) (1/11), high hyperdiploidy (2/9) and iAMP21 (0/3). MLPA did not detect any IKZF1Δ4-7 deletions that were not detected by PCR but did identify several samples with alternative IKZF1 deletions affecting different exons (see Table 1). By contrast, the PCR assay did detect six IKZF1Δ4-7 deletions undetected by MLPA, consistent with the higher sensitivity of this approach. Interestingly, three of these samples harboured alternative IKZF1 deletions in addition to IKZF1Δ4-7. In 70 (14%) cases, we observed a “faint” PCR band. Since the biological relevance of this was not clear, the ‘faint’ bands were not included in the final analysis. Interestingly the frequency of these “faint” bands was similar across all genetic subtypes: BCR-ABL1 (14%), B-other (15%), MLL (21%), low hypodiploidy (19%). We examined the impact of IKZF1Δ4-7 on achievement of CR, persistence of minimal residual disease (MRD) and overall survival (OS) and for all others...
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 GMA 05/93 and T-LBL/ALL 2004 protocols. Immunophenotype was determined by immunohistochemical staining and by FCM of cellular suspension obtained from lymph nodes (n=31), mediastinal mass (n=12) or nasopharyngeal/perinodal infiltration (n=2) by fine needle aspiration biopsy (FNAB), as well as of BM (n=10), PB (n=7) and pleural fluid (n=9). Disease subtype was defined according to WHO 2008 classification: pre-B, pro-B, pre-T, CD2+ or - (CD2+), cortical (CD1a+), medullary/mature (sCD3+). Recognition of pan-T cell CD antigen (pTag) expression included: CD1a, CD2, sCD3, CD4, CD5, CD8. ETP-T-LBL/ALL immunophenotype was defined as follows: absent (up to 5%) positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD5 expression, expression (25% positive cells) of 1 or more myeloid (CD13,CD33, CD15) or stem cell (CD34, HLA-DR) markers.

Results: Patient characteristics: ALL (BM+ >20%): n=26(37%), LBL: n=45(63%), BM+<20% involvement (LBL): 27%, age=35; years: 72%, males: 67%. Immunophenotype: pre-B: 21%, pre-T: 17%, cortical: 55%, medullary/mature (n>10% of pTag present): 0-3: n=3(27%), 0: n=21(53%), 3-7: n=2(3.5%). Most frequently expressed pTag were: CD7: 97%, CD5: 87%, CD7: 74%, CD1a: 58%. Myeloid markers: CD13/33/15 were expressed in 13%/26%/10% and stem cell markers: CD34 in 42% of pts. Overall, 19% (13/67) of the study population had ETP-T-LBL/ALL. CD1a- (9/13), but CD5 negative: 46% and CD5 weaker (20-71%): 54%, CD34/HLA-DR/CD15 in 100%/50%/75%/14% of ETP 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 of 0.99, 1.733 months, 5-year OS and DFS (95%(CI.), was 53% (0.42, 0.65) and 48% (0.36, 0.59), respectively. 5-year OS (95%(CI.)) for pts with sCD3 and CD1a expression as a pTag present was 64% (0.51, 0.77), 86% (0.51, 0.83) and 64% (0.5, 0.72) compared to 11% (0.03, 0.25), 32% (0.15, 0.49) and 27% (0.09, 0.452) for pts without CD2, CD13 and 0 or less pTag (P<0.001, 0.009 and 0.002), respectively. OS and DFS was dependent on WHO subtype (p<0.001/p<0.002), 5-year OS (95%(CI.): cortical: 69% (0.547, 0.82), pre-B: 48% (0.196, 0.78); maturity: 40% (0.24, 0.62), pro-B: 10% (0.072, 0.272). There was no significant difference in OS and DFS in pts with ETP vs non-ETP (P=0.198, 0.159). 5-year OS (95%(CI.): 31% (0.57, 0.559) and 55% (0.418, 0.685) for ETP and non-ETP pts, respectively. Among ETP pts, 4/13(31%) are alive, 3/4(75%) pts with pre-T and only 1/9((11%) with pro-T phenotype. 3/7 pts were rescued with allo-HCT (Figure 1).

Summary/Conclusions: Survival of T-LBL/ALL pts depends on CD1a and CD2 expression as well as on WHO subtype. ETP is a non-uniform category for pro-T/pre-T-cell origin. ETP phenotype was non-significant factor for OS/DFS (p>0.12/p>0.15) unless consistent with pro-T subtype (CD2+), only 1/9 pts alive. Pro-T (CD2-) is an ultra-high risk subtype of T-LBL/ALL and novel treatments are needed to improve pts outcomes.

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CLINICAL SIGNIFICANCE OF END OF INDUCTION MINIMAL RESIDUAL DISEASE IN ADULT PATIENTS WITH ACUTE LYMPHOBlastic LEUKEMIA IN COMPLETE REMISSION AFTER A SINGLE CHEMOTHERAPY COURSE


Background: In pediatric ALL end of induction minimal residual disease (E01 MRD) evaluated at day 29-33 after the first chemotherapy course is a primary determinant of outcome. The significance of E01 MRD in adult ALL is less clear.

Aims: To assess E01 MRD and its impact on survival and relapse rate in adult patients with Philadelphia-negative (Ph-) ALL in complete remission (CR) with a single chemotherapy course.

Methods: Induction chemotherapy for patients in the Northern Italy Leukemia Group 10/07 trial (ClinicalTrials.gov NCT-00795756). Blood in 2016;128:176-187. All patients were confirmed negative at week 10, contrary to only 7/28 (25%) with E01 MRD. Immunophenotype was determined by immunohistochemical staining and by FCM of cellular suspension obtained from lymph nodes (n=31), mediastinal mass (n=12) or nasopharyngeal/perinodal infiltration (n=2) by fine needle aspiration biopsy (FNAB), as well as of BM (n=10), PB (n=7) and pleural fluid (n=9). Disease subtype was defined according to WHO 2008 classification: pre-B, pro-B, pre-T, cortical (CD1a+), medullary/mature (sCD3+). Recognition of pan-T cell CD antigen (pTag) expression included: CD1a, CD2, sCD3, CD4, CD5, CD8. ETP-T-LBL/ALL immunophenotype was defined as follows: absent (up to 5%) positive cells) CD1a and CD8 expression, absent or dim (75% positive cells) CD5 expression, expression (25% positive cells) of 1 or more myeloid (CD13,CD33, CD15) or stem cell (CD34, HLA-DR) markers.

Results: Of 163 study patients, 139 were in CR after cycle 1 (85.2%), 95 had a sensitive molecular marker (68.3%) and 90 were successfully studied for E01 MRD. Median patient age was 39 years (range 17-67 years), 58.9% were male, 64.4% and 35.6% had B- and T-ALL, respectively, 44.4% were SR, 10% HR. 9,11,13,16,18,20; intrathecal MTX, Ara-C, Dexa on days 1 and 15; and G-CSF from day 4. CR was assessed on day 28 by means of bone marrow morphology and MRD evaluation (RO-PCR with case-specific probes, sensitivity ≥10-4).

E01 MRD was missing in 32 (19.6%) patients, whereas there was a risk of relapse (incidence (RI) and relapse-free survival (RFS), defining as favorable (i.e. negative) a response <10-4. In this risk-oriented protocol MRD-based risk stratification was deferred until after early consolidation (week 10), at which point standard- and high-risk (SR, HR) patients with MRD ≥10-4 and very HR patients with MRD study results were eligible to aleloigeneic stem cell transplantation.

Summary/Conclusions: Survival of T-LBL/ALL pts depends on CD1a and CD2 expression as well as on WHO subtype. ETP is a non-uniform category for pro-T/pre-T-cell origin. ETP phenotype was non-significant factor for OS/DFS (p>0.12/p>0.15) unless consistent with pro-T subtype (CD2+), only 1/9 pts alive. Pro-T (CD2-) is an ultra-high risk subtype of T-LBL/ALL and novel treatments are needed to improve pts outcomes.
Background: The outcome for older adults with acute lymphoblastic leukaemia (ALL) is unsatisfactory. The UKALL12/ECOG2993 study showed that high risk cytogenetic abnormalities, were common, as well as lower rates of complete remission (CR) and 5 year overall survival (OS) in those aged 55-65 years of age as compared to younger persons. There are few studies which focus on older patients with ALL, despite an increasing incidence with age.

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

Methods: UKALL60+ offers five ‘Arms’ to be decided by investigator and patient choice; Arm A= Philadelphia chromosome positive (Ph+), Arm B= Non-intensive (designed to be delivered primarily out of hospital), Arm C= Intensive, Arm D= Intensive + and Arm E= Registration only (in which treatment is at 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 a mix of hypo- & hyper-diploid. FISH studies were available for 50 pts: 10 (41%) had complex cytogenetics (≥5 abnormalities), 18 (38%) had a monosomy and 32 (60%) received Hyper-CVAD, concomitantly with rituximab in 11 (34%) pts, & TKIs in 9 (28%) pts. 21 (40%) pts received other regimens, of which 14 (67%) had asparaginase-based chemotherapy. Only 2 (4%) pts who received induction chemotherapy died within the first 60 days; both received Hyper-CVAD. Median number of cycles to achieve CR was 1 (1-8) with CR/CRi rate of 93%, & median time to CR1 was 34 days (19-459). 3 pts who underwent palliative chemotherapy achieved CR (all had Ph+ disease & received TKIs). 7 pts (13%) had primary induction failure. 50% of pts relapsed within a median time of 12.6 (3.6-72.8) months. Only 10 pts 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) months (p=0.0016). In a univariate analysis model which included multiple variables, only ECOG PS ≥2, WBC≥30,000, CDXN2A del, & CNS leukemia were statistically significant, however only CNS leukemia (p=0.0009) & WBC (p=0.0168) retained the statistical significance in multivariate mode, with a trend in CDXN2A del (p=0.06) (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.
Background: The outcomes 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. Treatment 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 did not of the transplanted cases, 6 are in CR and 2 have died due to complications, while 11 of the non-transplanted patients are in first CR, 3 have relapsed and 1 has died in first CR (p=n.s.). Of the 5 patients transplanted in second CR, only 1 is alive.

Figure 1.

Summary/Conclusions: This study further underlines the benefit of an induction based on a TKI alone. Since age holds strong prognostic significance, our results suggest that while chemotherapy followed by consolidation chemotherapy is the optimal choice for adults, in elderly cases dasatinib is more appropriate, since patients are often unfit to receive further chemotherapy. Finally, the advantage of SCT needs to be carefully redefined in the TKI era.

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

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

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

Results: Among 118 cases included, blasts of 92 patients (78.0%) were CD9+ (92% of CD9+expressing blasts). There were no significant differences in age, sex and white cell count between CD9+ and CD9- patients. Major cytogenetics subgroups were similarly distributed except for hyperdiploidy (all patients were CD9+: P=0.022) and ETV6-RUNX1 translocation (higher prevalence in CD9− patients: P=0.001). Significantly more CD9+ patients were stratified into the intermediate-risk group (P=0.044) and a higher proportion of CD9− patients was stratified into the high-risk group (P=0.025). Besides, CD9− patients had poorer prednisone response (P=0.014). The 5-year overall survival (OS) and relapse-free survival (RFS) rates of CD9+ patients were significantly lower than those in CD9− patients (P<0.029). Subgroup analysis revealed remarkably poorer outcomes in CD9+ patients of the high-risk group (P<0.045). A similar trend was also observed in patients of the intermediate-risk group but not in the standard-risk group. In univariate analysis, CD9 positivity, age <1 year, white cell count ≥100 × 10⁹/L and poor prednisone response were associated with lower RFS rate (P<0.001). In multivariate analysis, CD9 positivity (HR=4.0; P=0.019) and poor prednisone response (HR=3.9; P<0.015) remained as independent prognostic factors for lower RFS rates.

Summary/Conclusions: Our data indicate that expression of CD9 was significantly associated with inferior survival outcomes in pediatric B-ALL. The observation of the potential role of CD9 in pediatric B-ALL is most promising for patients in the intermediate-risk group, suggesting that CD9 expression could potentially be used in conjunction with other known prognostic factors for refinement of risk group stratification. Our study also lays the foundation for future development of CD9-targeted therapy for high-risk and relapsed/refractory pediatric B-ALL.
Results: Our series included 69 boys and 57 girls diagnosed with acute leukemia, with a median age of 6.1 years (range 0-17.4 years). We included 12 infant patients (<1 year old). Eighty-two (65%) patients had B-cell precursor acute lymphoblastic leukemia (BCP-ALL), 24 patients T-cell ALL and 20 patients had acute myeloblastic leukemia (AML). Globally, we found higher expression levels of class I HDAC isozymes (HDAC 1, 2, 3 & 8) in leukemic samples as compared to their non-neoplastic samples, as previously reported. Interestingly, some HDAC isoforms associated with specific genetic aberrations. Those patients with rearrangement of MLL (KMT2A) gene (n=18, including 9 BCP-ALL and 9 AML; 7 infants and 11 pediatric) had a significantly lower expression of HDAC3 (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 89% at 10 years for BCP-ALL and 55% for T-ALL patients (p=0.0035 & p=0.02, respectively, see Figure 1). Infants (n=12) had also a significantly lower expression of HDAC7 (p=0.043). In the BCP-ALL subgroup, the expression of HDACs did not predict outcome, and only CNS infiltration and leukocytosis were unfavorable risk factors for OS. Again, CNS+, high WBC count and presence of minimal residual disease (MRD) post-induction were predictive for worse event free survival (EFS). Although the number of cases is low and these results must be taken with caution, T-ALL patients with the highest expression of HDAC3 (upper quartile) significantly correlated with worse OS (94% vs 25%, p=0.001) and a trend towards worse EFS (89% vs 53%, p=0.06). The only significant risk factor for EFS in this subgroup was the presence of MRD after induction (p=0.003).

Figure 1.

Summary/Conclusions: We have observed a specific pattern of HDACs expression in pediatric patients with ALL rearrangement. Our study, if further confirmed, suggests that specific HDACi would potentially be a useful targeted treatment for pediatric patients with ALL 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 marrows (BM) by morphology and immunophenotyping, tissue biopsy (lymph node or mediastinal mass). This indicates flowcytometry is necessary in cases with <5% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as studies have confirmed, suggests that specific HDACi would potentially be a useful targeted treatment for pediatric patients with ALL rearranged leukemia.

Methods: A retrospective analysis of 42 cases of T-LBL with <25% blasts identified by morphology and immunophenotyping. T-LBL with <5% blasts in the peripheral blood (PB) and/or bone marrow in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as studies have confirmed, suggests that specific HDACi would potentially be a useful targeted treatment for pediatric patients with ALL rearranged leukemia.

Results: A retrospective analysis of 42 cases of T-LBL with <25% blasts in the peripheral blood (PB) and/or bone marrow in cases with <25% blasts identified by morphology. The identification of minimal disseminated disease in T-LBL is important as studies have confirmed, suggests that specific HDACi would potentially be a useful targeted treatment for pediatric patients with ALL rearranged leukemia.

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INOTUZUMAB OZOGAMICIN IN COMBINATION WITH LOW-INTENSITY CHEMOTHERAPY (MINI-HYPER-CVD) AS FRONTLINE THERAPY FOR OLDER PATIENTS WITH ACUTE LYMPHOBластIC LYMPHEMIA: UPDATED RESULTS FROM A PHASE III 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, 83% 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.
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=96%). Best response was CR in 36 pts (84%), CRp in 5 (12%) and CRi in 1 (2%). MRD negativity by 6-color multiparameter flow cytometry was achieved in 31 of 41 evaluable pts (76%) on day 21 and in 44 of 46 evaluable pts (96%) within 12 weeks of treatment. The median follow-up was 24 months (range, 1.5-55 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 21 pts remain on treatment (consolidation, n=3; POMP maintenance, n=19), and 5 pts have completed all therapy. The 3-year continuous CR and OS rates were 72% and 54%, respectively. Compared to a historical cohort of 79 older pts treated at our institution with hyper-CVAD ± rituximab, mini-hyper-CVD+InO resulted in significantly improved OS (3-year OS rate: 54% vs 31%; median OS not reached versus 16 months; P=0.007).

Treatment was overall well-tolerated. The median times to platelet and ANC recovery in cycle 1 were 22 days (range, 11-91 days) and 16 days (range, 0-49 days), respectively. In cycles 2-8, the median times to platelet and ANC recovery in cycle 1 were 22 days (range, 11-91 days) and 16 days (range, 0-49 days), respectively. Compared to a historical cohort of 79 older pts treated at our institution with hyper-CVAD ± rituximab, mini-hyper-CVD+InO resulted in significantly improved OS (3-year OS rate: 54% vs 31%; median OS not reached versus 16 months; P=0.007).

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

### Acute myeloid leukemia - Biology 1

**P171 RECURRENT MYB REARRANGEMENT IN BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM**

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

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

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

**Results:** We identified a recurring gene rearrangement that involved the MYB proto-oncogene in all five pediatric patients (100%) and four of nine adult patients (44%) with BPDCN. The resulting fusion genes included MYB-ZAP7 (four patients), MYB-PLEKHO1 (three patients), MYB-DCPS (one patient), and MYB-MIR2134 (one patient), none of which have been previously reported to our knowledge. The translocations corresponding to these fusions were not detected by the metaphase analysis except in one patient with t(1,16), who harbored MYB-PLEKHO1. These fusion genes were detectable at diagnosis and relapse but not at remission.

Fluorescence in situ hybridization (FISH) analysis efficiently detected the breaking apart of MYB in formalin-fixed, paraffin-embedded sections. Consequently to the rearrangement, the negative regulatory domain of MYB was truncated, leading to constitutive MYB transcriptional activation, as described in other malignancies. Exogenous MYB-PLEKHO1 expression in HEK 293T cells led to the upregulation of several known downstream MYB targets.

Gene set enrichment analysis also confirmed the activation of MYB target gene sets. The identified significantly upregulated genes included cell surface molecule-encoding genes such as NCAM1 (also termed CD56), CD68, S1PR1, and CXCR4, possibly providing targets for antibody-mediated anticancer therapies. We performed whole-exome sequencing of paired tumor– normal DNA sets. At diagnosis for four pediatric patients, which revealed a total of 91 (6–45 per patient) somatic mutations, a relatively large number compared with other pediatric cancers. However, no driver mutations were identified from the existing literature and database entries; only one missense mutation, KMT2D p.Cys1403Gly, was present on a driver gene, although this exact mutation had not been previously reported. Furthermore, we performed targeted sequencing covering genes associated with hematological malignancies in the remaining 10 patients. Consequently, children were not found to carry any identifiable driver mutations, whereas all adult patients harbored at least one point mutation in genes such as TET2, ASXL1, IKZF1, ZRS2, NRAS, and EZH2, most of which were reported to be mutated in BPDCN and myeloid malignancies.

**Summary/Conclusions:** We identified a high frequency of MYB rearrangements that promoted the MYB transcriptional activity in BPDCN. MYB split FISH analysis can constitute a valuable diagnostic tool for detecting MYB rearrangements. We expect that our findings provide critical insights regarding BPDCN pathogenesis and contribute to molecular biology-oriented diagnostic techniques and molecular-targeted therapies for this intractable malignancy.
Background: The branched chain amino acids (BCAAs) valine, leucine, and isoleucine are essential AAs for the human body. The activity of BCAA metabolism involves the regulation of cytoketoglutarate (αKG) and α-amino groups from BCAAs to α-ketoglutarate (αKG), respectively. Inhibition of nuclear export is critical for the leukemic phenotype. The most frequent NPM1 mutation is a heterozygous 4bp insertion in exon 12 (mutA).

Methods: To elucidate the mechanistic link between BCAAT1 function and epigenetic deregulation in leukemia stem cells (LSCs) and consequences on clinical outcome.

Methods: High-resolution proteomics of LSCs, Knockdown and overexpression of BCAAT1 in AML patient samples and AML cell lines, Gene set enrichment analysis, BCAA tracing experiments, Xenotransplantation, Metabolomics, RNA-sequencing arrays, correlative and mechanistic link to clinical data sets.

Results: We performed high-resolution proteomics analysis of human acute myeloid leukemia (AML) stem cell (LSC) and non-LSC populations, which have been functionally validated by xenotransplantation into NSG mice, and we found the BCAA pathway enriched in AML and overexpressed in LSCs. We show that BCAAT1, which transfers α-amino groups from BCAAs to α-ketoglutarate (αKG), is a component of the nuclear export machinery (NPM1) and its role is hindered by the presence of the branched chain amino acid (BCAA) cycle αKG (a core essential factor for αKG-dependent dioxygenases such as EGLN1 and the TET family of DNA demethylases). Knockdown (KD) of BCAAT1 in leukemia cells caused accumulation of αKG resulting in HIF1α protein degradation mediated by EGLN1. This resulted in a growth and survival defect and abrogated leukemia-initiating potential. In contrast, overexpression (OE) of BCAAT1 in leukemia cells decreased intracellular αKG levels and caused DNA hypermethylation. BCAAT1 high AML samples displayed a DNA hypermethylation phenotype similar to IDHmut cells, in which KD is inhibited by overexpression of the oncometabolite 2-hydroxylglutarate. High levels of BCAAT1 were strongly correlated with IDHmut (IDHmutTET2mut) and, but not IDHmut TET2mut AMLs. Gene sets characteristic for IDHmut AMLs were enriched both in IDHmutTET2mut- and TET2mut high patient samples and in BCAAT1-OE leukemia cells. BCAAT1 high samples showed enrichment for LSC signatures and paired sample analysis revealed a significant increase of BCAT1 levels upon relapse of the disease.

Summary/Conclusions: In summary, BCAAT1 reduces dioxygenase activity by limiting intracellular αKG, thus linking BCAA catabolism to HIF1α stability and DNA hypermethylation. Our results suggest the BCAA-BCAT1-αKG pathway as a therapeutic target to compromise LSC function in IDHmutTET2mut AML patients.
Summary/Conclusions: Conclusions: HOXB-AS3 is strongly associated with NPM1 mutations in AML. HOXB-AS3 interacts with EB1p1 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 EFFICIENCY AND WELL-TOLERATED IN ACUTE MYELOID LEUKEMIA

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Background: Identification of a chemotheraphy-free option for acute myeloid leukemia (AML) represents a highly desired and important research objective. Perturbation of cell survival is an essential hallmark of cancer now amenable to precision targeting by small molecule BH3-mimetics able to inhibit pro-survival BCL-2 (e.g. Souers et al Nat Med 2013 and Roberts et al., NEJM 2016), BCL-XL (Lessene et al., Nat Chem Biol, 2013) and MCL1 (Kotschy et al, Nature 2016). We hypothesise 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) from Guillaume Lessene (WEHI). Primary AML cells were obtained from patients providing informed consent. For in vivo experiments, NSG; NOD.Cg-Prkdcscid Il2rgtm1Wjl (NRGS) or NOD/ Rag2−/−Il2rgtm1Wjl (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).

Figure 1. (A) Loewe Score (0 = Additive, 1 > Weak Synergy, 2 > Synergy, 5 > Strong Synergy) in AML cell lines (Lehar, Nat. Biotech 2009). (B) LC50 of primary AML after 48hr treatment (C) NS mice engraffed with MV4;11 AML and treated with i) vehicle, ii) S55746 100 mg/kg (x 4 wks), iii) S63845 25mg/kg IV (x 4 wks) or iv) combined S55746 and S63845. (D) CD45+ staining of NRGS sternums showing 2 representative examples of PDX AML one week after treatment with i) vehicle x5d, ii) S55746 100 mg/kg/d x 5d, iii) S63845 25mg/kg IV x 2d and iv) S55746+S63845.

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 wild type NPM1 and MLL-AF4. Patient-derived xenografts demonstrated rapid reduction of established AML in the bone marrow one week of treatment with S55746 and S63845 (Figure 1D). Safety and tolerability of this approach was confirmed using normal CD34+ stem and progenitor cells in short-term cell culture (48h) and long-term (2-3 weeks) clonogenic assays and from historical and biochemical examination of mice receiving treated for up to 8 weeks at doses shown to be highly efficacious against AML.

Summary/Conclusions: Dual BH3-mimetic targeting of BCL-2 and MCL1 induces rapid and synergistic 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 first time, that dual pharmacological targeting of both BCL-2 and MCL1 represents a novel approach to treating AML without need for additional chemotherapy and with an acceptable therapeutic safety margin. Our results support the translational investigation of dual BH3-mimetic targeting of BCL-2 and MCL1 in the clinic for the treatment of patients with AML.

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THE PML/PLIC2/PLIC5A 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 oncopogene 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 P56 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 leukaemia (p<0.0001), with a reduced latency period (p=0.008). Moreover, response to targeted therapy with ATRA significantly increased the survival period (p=0.008). Using DNA repair reporter assays, we demonstrated that DNA repair via both non-homologous end joining (NHEJ; p=0.01) and homologous recombination (HR; p=0.006) pathways was less efficient in PmlC62A/C65A primary cells than in PmlWT cells. Importantly, using a PML-RARA-inducible cell line, comparable defects in the NHEJ and HR pathways, which were PML-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 analysing Foci size and γH2AX foci formation, was not affected. None of the DNA repair players analysed (e.g. Blm, Rad51 and S3BP1) 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 significant reduction in size post IR in PmlC62A/C65A compared to PmlWT, with expression of IR, 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 Pmi NB to the effectiveness of DNA damage repair processes, and the manner in which their disruption mediated by the PML-RARα oncoprotein can assist APL pathogenesis.

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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 we aim to study these mutations in the context of their respective roles in dictating clinical courses 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 representing 148 mutation combinations- reproducibly transformed LSK cells with distinct clonal output. Transplantation of in vitro immortalized clones yielded robust engraftment with multi-lineage contributions in mice but no overt leukemia was detected, indicating that induced mutations select for a preleukemic state in vitro. We thus tested every oncogene/tumor suppressor pool from the in vitro setting in a murine bone marrow transplantation model with freshly transduced LSK cells which resulted in robust induction of leukemia. Analysing the mutational spectrum of derived clones we were able to raise a list of potential partners cooperating with EzH2 loss, which highlighted Nf1 (Ras-signaling), loss of Dmnt3a, and loss of Runx1 as cooperating partners, whereas loss of cohesin complex subunits (Smc3, Stag2) seems to be dispensable during the induction of EzH2-loss guided leukemogenesis. To define oncogenic dependencies in myeloid malignancies with PRC2-loss we analysed gene expression spectra of the generated samples. While in vitro transduced clones presented with distinct expression signatures clearly separating from controls a partially overlapping expression signature could be established. Through identification of these expression signatures and the resulting gene expression signature, which will be validated in a CRISPR-Cas9 knock-out screening we aim to identify novel therapeutic targets in AML.

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

P178

Abstract withdrawn.

P179

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

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

Methods: Whole exome sequencing (WES) of 12 diagnostic AML samples with the matched germ-line controls (T cells or buccal swab) was performed. Leukemia-specific mutations were identified according to specific criteria (Allele Depth >20, Support >20, Score >80, SIFT-coefficient >0.20, coverage >10 reads, support >2 reads, and GMAF <0.05) and validated. Identified leukemia-specific mutations were tracked in different cellular compartments (T- and B-cells) as well as in single HSC derived from diagnostic AML samples. To test the functional properties of pre-leukemic HSC in vivo, we transplanted bulk AML in NOD/SCID-IL2γnull (NSG) mice and analyzed human subpopulations (myeloid and lymphoid) of multi-lineage engrafted animals for the presence of leukemia-specific mutations.

Results: WES identified 64 AML-specific mutations. Most cases (8 out of 12) showed 4-6 AML specific mutations per sample (1-18 mutations/AML) including mutations in genes that are recurrently mutated in leukemia yet (NRAS, KIT). Tracking of AML-specific mutations in non-leukemic T- and B-cells showed that some AML mutations like DNM3TA, 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-compartment suggesting that these animals were engrafted from functionally normal pre-leukemic HSC. To reconstruct the sequence of pre-leukemic mutations single-cell HSC were sequenced 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 DNM3TA mutation in myeloid and lymphoid cells, whereas NPM1 and FLT3-ITD mutations were only detectable in leukemia cells proving the pre-leukemic status of DNM3TA in this case. In another patient we found DNM3TA and IDH2 in T- and B-cells whereas TRIP13 and RPL57A mutations were only detectable in leukemia cells. By analyzing colonies from single cell HSC we were able to detect complex pre-leukemic hierarchies with one example in which a ZNF536 mutation could be identified as initiating event that hasn’t been described in leukemia yet.

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

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THE ESSENTIAL ROLE OF THE ENHANCERS OF POLYCOMB EPC1 AND EP2C IN MLL-AF9 ACUTE MYELOID LEUKAEMIA IS A ‘COMPLEX’ STORY
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Background: The Enhancers of Polycomb (EPC) proteins EPC1 and EPC2 are essential for the survival of MLL-AF9 rearranged acute myeloid leukemia (AML). Most importantly, loss of EPC1 or EPC2 in MLL leukemia stem cells, but not normal hematopoietic stem cells and progenitor cells, leads to the induction of cellular apoptosis. To date little is known about the functional contribution of EPC1 and EPC2 in AML. EPC1 and EPC2 are two major chromatin modifying complexes encompassing opposing epigenetic activities and both are known to be deregulated in AML. A recent study identified a unique subset of human malignancies due to the homologous chromatin regulatory proteins EPC1 and EPC2 in AML in search for novel therapeutic targets.

Methods: Mass spectrometry (MS) analysis was performed on immunoprecipitated protein using EPC1 antibody from human THP1 MLL-AF9 AML cell line. Chromatin immunoprecipitation (ChIP) was performed using HighCell ChIP Kit and iPure kit V2 (Diagenode) followed by NextSeq500 Illumina sequencing in THP1 cells. ChIP enriched regions were identified using SICER peak calling and ChIPpeakAnno. Lentiviral supernatants were prepared and THP1 cells were infected with viral particles containing pLKO.1 puro lentiviral vector expressing shRNAs. RNA was extracted 72 hr following lentiviral transductions and whole transcriptome sequencing was performed. DESeq2 was used for differential expression analysis.

Results: MS analysis identified the core NUA4 complex components (TIP60, ING3, RUVBL1, RUVBL2, EP400 and DNA1) and also revealed additional DNA and histone modifying 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, ING3, RUVBL1, RUVBL2, EP400 and DNA1) and also revealed additional DNA and histone modifying shRNAs. RNA was extracted 72 hr following lentiviral transductions and whole transcriptome sequencing was performed. DESeq2 was used for differential expression analysis.

Summary: The role of the EPC proteins in AML is not yet clear. EPC1 and EPC2 are essential for the survival of AML stem cells and their loss leads to the induction of cellular apoptosis. Further work is needed to identify how EPC1 and EPC2 contribute to the survival of AML stem cells.
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 monoyctic 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 PRC-associated complexes which are essential for the maintenance of an AML epigenetic signature and an aberrant transcriptional profile that supports leukemia stem cell survival. We have identified and characterized the EPC complex components in human AML. Additionally, we have refined a subgroup of PRC target genes that are regulated by the EPC complex which represent potential novel therapeutic targets in human AML. Overall we present a comprehensive analysis of the aberrant epigenomic landscape of THP1 MLL-AF9 AML cells in relation to EPC1 and EPC2 and provide new insight into their deregulated role in AML.

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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 governing 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*AML cells. Mice were divided into control, venetoclax (25 mg/kg, i.p.), ruxolitinib (50 mg/kg BID, p.o) and 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 could induce resistance to venetoclax similar to CM that we showed earlier to reduce sensitivity to BCL2 inhibitors. To identify the impact of stroma-derived factors on cellular signaling we stimulated AML patient cells with CM and analyzed the phosphorylation of STAT3, STAT5, ERK and AKT. Compared to control conditions, CM rapidly induced phosphorylation of STAT5 in primary AML cells. When the effect of individual cytokines was tested, we noted that GM-CSF and G-CSF alone could mimic the effect of CM on cellular signaling. Gene expression data showed the receptor for GM-CSF (CSFR2A) 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, a downstream effector of JAKs, we tested a combination of venetoclax and JAK1/2 inhibitor ruxolitinib. We found that ruxolitinib potentiated sensitivity to venetoclax when tested with AML patient cells in HS-5 CM and in co-culture and transwell assays. Significantly, the combination was more effective at reducing tumor burden in a xenograft mouse model of AML than either drug alone.

Summary/Conclusions: In conclusion, our data demonstrate that BM secreted soluble factors drive cytoprotection against BCL2 antagonist venetoclax that can be overcome by combined blockade of JAK/STAT and BCL2 pathways with ruxolitinib and venetoclax in ex vivo co-culture models and in vivo in an AML mouse model.

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IDENTIFICATION OF NOVEL GENE FUSIONS IN ACUTE MYELOID LEUKEMIA WITH COMPLEX KARYOTYPE USING RNA SEQUENCING
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Background: Acute myeloid leukemia with complex karyotype (CK-AML), defined as having ≥3 acquired cytogenetic aberrations in the absence of WHO-defined recurring translocations or inversions, represents about 15% of
adult AML cases. Despite having poor outcomes, CK-AML is the least understood molecular lesion, except for the finding that about two-thirds of cases carry TP53 alterations. In particular, because cytogenetic alterations appear to be distinct among different patients, it is unclear whether they are cause of leukemogenesis, or merely reflect a state of genomic instability.

**Aims:** We have hypothesized that cytogenetic aberrations in CK-AML create genetic lesions that are recurrent across patients, notwithstanding distinct cancer genes that contribute to leukemogenesis in individual patients. Therefore, 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 fusion junctions) that are independent across patients, along with a novel fusion prediction method. Identified gene fusions were in part independently validated by array-based genomic profiling and/or long range PCR obtained by use of long-read Oxford Nanopore sequencing technology.

**Results:** We identified 54 gene fusion events in 30 of the 65 cases (46%) with up to four fusions per case. All fusions are supported by 10-50× junction-spanning paired reads, and the 5' and 3' fusion junctions are independently predicted as fusion breakpoints in genomic DNA from array-based genomic profiling and/or long range PCR, respectively. About 35% of the fusions were in-frame, encoding chimeric proteins. The remaining encodes either C-terminally truncated 5' fusion partners, or else N-terminally truncated (or rarely full-length) 3' fusion partners. In 65% of cases, the 5' partner 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-MN1) were previously reported in AML. The most frequently affected genes were RUNX1 (n=5), KMT2A, and MECOM (n=3 each). Identified gene fusions were categorized into six functional fusion clusters. Many of the fusions contained at least one known AML gene (n=16; e.g. RUNX1, MECOM, DEK, ETV6, KMT2A) together with a novel fusion partner, clearly suggesting pathogenic relevance. Other fusions were predicted to disrupt known tumor suppressors (n=4; e.g. TP53, SMAD4) or to activate known oncogenes (n=5; e.g. MLL2). Chimeric fusion proteins encode chimeric proteins of unclear pathogenic relevance, but that could nonetheless encode novel epitopes created by the fusion junction (n=26).

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

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**Figure 1.**

**Summary/Conclusions:** the biomarker H3K27me3 HIST1high is correlated with a better LFS and OS 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 HIST1 genes that could explain treatment failure.

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**References:**


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**Figure 1.**

**Summary/Conclusions:** the biomarker H3K27me3 HIST1high is correlated with a better LFS and OS 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 HIST1 genes that could explain treatment failure.

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**References:**


ity of protein expression was normalized to actin. That ratio of phosphorylated protein to total protein for FLT3 and STAT5 was determined and normalized to that observed in the D835Y mutation as a positive control. A value of >10% pFLT3 was considered positive. All mutations that resulted in FLT3 phosphorylation were subsequently evaluated for inhibition by crenolanib and quizartinib following 60-minute exposure to the compounds.

Results: All ITD, non-ITD and non-AML FLT3 mutations were evaluated for autonomous FLT3 and STAT5 phosphorylation. Eleven mutations resulted in pFLT3 and pSTAT5, including 4 mutations with >50% pFLT3. All mutations that demonstrated aberrant pFLT3 also had aberrant pSTAT5, however a direct correlation of pFLT3 and pSTAT5 was not always observed. Overall, 87% (n=86 patients) of all non-ITD mutations evaluated resulted in autonomous FLT3 activation. Excluding D835 mutations, 64% (n=39) of patients harbored an activating mutation. Many of the mutations that were not found to be activating had the lowest prevalence, often present in only one patient. Evaluation of inhibition of pFLT3 and pSTAT5 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 exclusively sensitive to crenolanib, with 9 of 10 mutations tested demonstrating an IC50 of pFLT3 inhibition ≤5.6 nM. Inhibition of downstream kinases is necessary for optimal efficacy of any FLT3 inhibitor and phosphorylation of STAT5 was potently inhibited by crenolanib in all cases. Quizartinib inhibited pFLT3 and pSTAT5 with an IC50 range of 1.8-151.7 nM and 1-33.9 nM respectively, demonstrating less effective inhibition specifically at mutations including D835Y, D839E, N670K, 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/835 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 LEUKAEMIA

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Background: The selective Bcl-2 inhibitor Venetoclax (ABT-199) has shown potential as a single agent in 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 Keap1 in the cytoplasm thereby Nrf2 enters the nucleus and binds to antioxidant response element sequences in the promoters of various genes. Nrf2 pathway activation has been shown to mediate chemoresistance in various cancers including AML. Low ROS levels have been shown to be a hallmark of leukemia stem cells and are critical to their self renewal capacity. In this study, we examined whether Nrf2 inhibition is an additional mechanism responsible for the marked antileukemic activity in AML seen with the combination of HMAs and venetoclax.

Aims: To determine the effect of venetoclax on ROS levels after HMA exposure in AML cells and to examine the effect of Bcl-2 inhibition on ROS 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, which results in dissociation of the Nrf2 complex and targets Nrf2 to ubiquitination and proteosomal degradation.

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 translocalization of Nrf2 (Figure 1) and induction of downstream antioxidant enzymes including HO-1 and NQO1. Immunofluorescence studies confirmed the inhibition of nuclear translocation of Nrf2 by venetoclax. Immunoprecipitation studies indicated that Bcl-2, Keap1 and Nrf2 associate in a protein complex in the cytoplasm and that treatment with venetoclax leads to dissociation of Bcl-2 from the Nrf2/Keap1 complex and targets Nrf2 to ubiquitination and proteosomal degradation.
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Background: The complex pathogenesis of cancer often necessitates combination therapies to optimize patient benefit. Thus, we investigated preclinical combinations of SY-1425 (tamibarotene) and other agents to build on the monotherapeutic strategy with SY-1425 in biomarker selected AML and MDS patients (Phase 2 study, NCT02807558). Based on the RARA 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 and HMAs and their novel agent in AML and MDS. We hypothesized that the HMA azacitidine could prime AML cells for SY-1425 mediated reprogramming by relieving aberrant methylation of RARA 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 CD38 mRNA expression. Antibody dependent cell-mediated cytotoxicity (ADCC) was tested in an ex vivo co-culture model of human NK cells and AML cell lines.

Results: SY-1425 acts as a repressive transcription factor until bound by SY-1425 leading to potent, targeted activation of myeloid genes. HMAs can further prime this activation by depleting repressive methylation of these target genes. The combination of SY-1425 and azacitidine showed synergy in RARA-high AML cell lines, but not in RARA-low AML cell lines, with combination indices less than 0.5. Co-administration in a RARA-high AML PDx demonstrated superior reduction of tumor burden (<1% detectable tumor cells) vs either treatment alone (49% reduction 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). RARA binds directly to the CD38 locus and induces H3K27 acetylation at this locus via recruitment of the mediator complex, CBP, and mRNAs 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. AML cell lines treated with SY-1425 and DARA were six fold more sensitive to NK cell mediated ADCC compared to single agent controls and exhibited a 5-10 fold increase in NK cell dependent activation measured by IFNγ secretion.

Summary/Conclusions: The RARA biomarker dependent synergy with azacitidine and SY-1425 is hypothesized to work through hypomethylated based priming of myeloid differentiation by SY-1425 agonism of formerly repressed RARA target genes. Since CD38 is one of the most strongly induced RARA 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.

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

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Background: FLT3 internal tandem duplication (ITD) mutations account for ~20-25% of adult AML cases and are associated with worse prognosis. Although FLT3 inhibitors show clinical activity, relapse occurs quickly. Veneto- clax is a potent, selective inhibitor of the anti-apoptotic protein BCL-2 that demonstrated monotheraputic activity in relapsed/refractory AML (ORR 19%); however, no activity was seen in FLT3 mutant cases (Konopleva, Can Disc and Ant 2013). In addition, FLT3 ITD regulation is complex between NRPs and RPs. Taken together, our preliminary data suggests that already at diagnosis, AML cells display an epige- nomic fingerprint associated with the development of AML relapse during the course of disease. We are currently validating these data.

Methods:

SY-1425 could induce sensitivity to the anti-CD38 therapeutic antibody daratumumab (DARA) and that strong upregulation of the maturation marker CD38 in AML cells by SY-1425 mediated reprogramming by relieving aberrant methylation of RARα target genes. Since CD38 is one of the most strongly induced RARα targets in response to SY-1425, AML blasts can be sensitized to DARA in combination with quinza- timin, a potent FLT3 inhibitor, resulted in synergistic anti-tumor effects in FLT3-ITD+ AML models.

Results: Sensitivity to venetoclax was initially assessed in vitro. Dose depend- ent growth inhibition and induction of apoptosis was observed in the MV4;11, Molm13 and HL60 cell lines following 48hr venetoclax treatment, with the MV4;11 cell line most sensitive. Modulation of BCL-2, BCL-XL and MCL-1 expression by FLT3 inhibition was determined following 8-24hr treatment with quinza-tinib. Quinza-tinib reduced BCL-XL and MCL-1 protein, but not BCL-2, in the FLT3-ITD+ cell lines. Quinza-tinib had no effect on expression of these three proteins in the QT3 wt cell lines. In the FLT3 wt cell lines, the combination of SY-1425 and venetoclax in vitro, cell lines were treated for 48hrs with venetoclax, quinza-tinib or the combination. Combination treatment led to significant reduction in prolif- eration and increased apoptosis in the FLT3-ITD+ cells compared to either single agent. FLT3 wt cell lines were sensitive to quinza-tinib as a single agent however induction with venetoclax was not synergistic. The combination of quinza-tinib and venetoclax showed synergy in the MV4;11 xenograft model. Together, this data underscores the dependency of these cells on the FLT3-ITD mutation for growth and its use as a predictive biomarker of veneto- clax resistance. To determine the dependency of FLT3-ITD+ cells on other anti-apoptotic proteins, cell lines were treated with selective antagonists to BCL-XL (A1331852) or MCL-1 (A1210477) in combination with venetoclax. Treatment with either antagonist in combination with venetoclax reduced the growth of the cell lines compared to venetoclax alone. However, co-inhibition of BCL-XL or MCL-1 and FLT3-ITD did not reduce cellular growth compared to quinza-tinib alone, indicating that maximum anti-tumor responses may be achieved when all three anti-apoptotic proteins are targeted.

Summary/Conclusions: Quizartinib treatment in FLT3-ITD+ AML models decreased expression of the anti-apoptotic proteins BCL-XL and MCL-1 and synergized with venetoclax in vitro and in vivo at clinically relevant doses for each compound. These data suggest that co-targeting FLT3-ITD with selective inhibitors and BCL-2 with venetoclax induces apoptosis to a greater extent than FLT3 inhibition alone. Importantly, our preclinical data supports further clinical investigation of this combination to treat FLT3-ITD+ AML.

P189

SPECIFIC TARGETING OF ACUTE MYELOID LEUKEMIA STEM CELLS BY INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN 7

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Background: Only 30-40% of acute myeloid leukemia (AML) patients survive five years after diagnosis. This extreme poor prognosis is mainly caused by treatment failure due to chemotherapy resistance. Leukemic stem cells (LSCs) are thought to be major determinants of AML recurrence due to their potential for self-renewal and chemotherapy resistance. LSCs co-reside with normal CD34+/CD38-, hematopoietic stem cells (HSCs) in the AML bone marrow. Increasing the dose of chemotherapy drugs may eliminate these chemotherapy resistant cells, however will inevitable result in the non-specific elimination of HSCs, delaying or even preventing the recovery of normal hematopoiesis after therapy. To significantly improve the outcome of AML patients, the discovery of alternative therapies that specifically eliminate LSCs while sparing HSC are urgently needed. To develop such therapies, accurate quantification of genes differentially expressed between LSCs and HSCs and between LSCs and the AML bulk is crucial.

Aims: To identify specific therapeutic strategies that have the potential to elim-inate AML relapse-initiating cells.

Methods: We generated gene expression profiles of HSCs, LSCs and leukemic progenitors all derived from the same AML bone marrow and identified Insulin growth factor binding protein 7 (IGFBP7) as one of the top differentially expressed genes. As low IGFBP7 expression is a feature of LSCs, we hypothesized that...
decreased expression of IGFBP7 might be associated with decreased chemotherapy sensitivity. To this end, we generated cell lines with IGFBP7 knockdown and subjected the cells to chemotherapy. Furthermore, to test whether increasing the IGFBP7 levels might be a strategy to deplete leukemic (stem) cells, we overexpressed IGFBP7 in or added recombinant human IGFBP7 (rhIGFBP7) to primary AML cells and measured clonogenic capacity, differentiation and cell survival in vitro. To study the effect of IGFBP7 on AML cell survival and engraftment potential in vivo, primary AML cells were transplanted into immune deficient mice and the mice were subsequently treated with rhIGFBP7. To study the effect of rhIGFBP7 on LSC survival, human AML cells derived from the first transplanted mice were re-transplanted into secondary recipients and engraftment and survival of the mice were monitored.

Results: Knockdown of IGFBP7 results in reduced sensitivity to chemotherapy and comparing matched diagnosis and relapsed AML samples showed that IGFBP7 expression is frequently downregulated at relapse, suggesting a survival advantage of IGFBP7low AML cells during chemotherapy treatment. Importantly, enhancing cytoplasmic or extracellular IGFBP7, by overexpression or addition of rhIGFBP7, resulted in induction of differentiation and apoptosis, increased sensitivity to chemotherapy and inhibited AML blast and leukemic stem/progenitor cell survival in vitro and in vivo. IGFBP7 had no influence on the survival of normal hematopoietic (stem) cells. Moreover, treatment with rhIGFBP7 can add to chemotherapy treatment by elimination of chemotherapy resistant refractory AML (stem) cells.

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

Acute myeloid leukemia - Clinical

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

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

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

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

Results: 32 adult BPDCN patients received SL-401 in stage 1 (n=9) and stage 2 (n=23), including 19 first-line and 13 R/R patients. Stage 3 patients will be reported separately. Median age was 72 years (range: 30-85 years). In stage 1, 12 ug/kg was the highest tested dose for BPDCN; MTD was not reached in BPDCN. Median follow-up was 4.3 months (range: 0.2-22.9 months). ORR of 84% (27/32) was observed in all patients: 96% (18/19) in first-line and 69% (9/13) in R/R. 88% (14/16) of first-line patients treated at 12 ug/kg had a complete remission (CR; n=10), CR with incomplete hematologic recovery (CRI; n=1) or clinical CR (CRC; residual skin disease; n=3) based on investigator assessment. 56% (9/16) of these patients were progression free for 4 to 22.9 months (ongoing), including 3 patients on SL-401 in remission for 18 months (ongoing) and 7 patients who were bridged to stem cell transplant (SCT; 3 acute SCT and 4 allo-SCT). A R/R patient was also bridged to allo-SCT. Overall, most common ≥Grade 3 treatment-related AEs were transaminase elevation (22%) and thrombocytopenia (16%). Safety precautions, including daily monitoring of albumin and body weight during study drug infusions, have been implemented to minimize risk of severe capillary leak syndrome (CLS). Three patients had Grade 5 CLS: BPDCN (7 ug/kg); R/R AML (16 ug/kg); BPDCN (12 ug/kg) out of 118 patients who received SL-401 across all trials and regimens; 3/89 (3.4%) patients of which were enrolled in this clinical trial.

Summary/Conclusions: SL-401 continues to demonstrate single agent activity, including multiple CRs, in patients with BPDCN, with 25% (8/32) of patients bridged to SCT after a major response from SL-401. SL-401 side effect profile consists largely of transaminitis and thrombocytopenia. CLS can be fatal. Side effects have generally tended to decrease in frequency and severity with increasing cycles. Updated data, including detailed safety analysis across all ongoing SL-401 studies will be presented at the meeting.

P192
PROGNOSTIC IMPACT OF SOMATIC MUTATION CLEARANCE IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA

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

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

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

Results: In the pre-treatment samples, we detected 597 mutations in 78 genes in 87 (92%) patients. In the matching CR samples, 62 (10%) and 82 (14%) mutations persisted at VAF>2.5% and ≥1%, respectively, which corresponded to 43 (49%), 34 (39%), and 30 (34%) patients achieving MC2.5, MC1.0 and CMC, respectively. Table 1 shows the differential patterns of MC based on the mutations and pathways. Mutations associated with clonal hematopoiesis of
indeterminate therapy (CHIP), DNA methylation, and splicing pathways had low rate of MC, whereas mutations in transcription factors like RUNX1 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 MC2 (median 31.2 vs 12.5 months, P=0.049) had significantly better relapse-free survival (RFS).

<table>
<thead>
<tr>
<th>Gen</th>
<th>MC2 (%D)</th>
<th>MC1 (%D)</th>
<th>CMC (%D)</th>
<th>Pathway</th>
<th>MC2 (%C)</th>
<th>MC1 (%C)</th>
<th>CMC (%C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNMT1</td>
<td>27%</td>
<td>17%</td>
<td>14%</td>
<td>CHIP associated</td>
<td>33%</td>
<td>24%</td>
<td>22%</td>
</tr>
<tr>
<td>TET2</td>
<td>100%</td>
<td>96%</td>
<td>96%</td>
<td></td>
<td>99%</td>
<td>99%</td>
<td>98%</td>
</tr>
<tr>
<td>TET1</td>
<td>19%</td>
<td>19%</td>
<td>19%</td>
<td>RTK pathway</td>
<td>88%</td>
<td>87%</td>
<td>86%</td>
</tr>
<tr>
<td>TET3</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>Transcription Factors</td>
<td>88%</td>
<td>87%</td>
<td>86%</td>
</tr>
<tr>
<td>CEBPA</td>
<td>100%</td>
<td>89%</td>
<td>89%</td>
<td>Chromatin-Remodeling</td>
<td>67%</td>
<td>65%</td>
<td>65%</td>
</tr>
<tr>
<td>EZH2</td>
<td>38%</td>
<td>44%</td>
<td>39%</td>
<td>Splicing</td>
<td>35%</td>
<td>17%</td>
<td>17%</td>
</tr>
</tbody>
</table>

Table 1.
Methods: multiple LSC markers on the outcome of AML patients.

Institution and eligible for intensive chemotherapy were enrolled from September 2010 to March 2016. We excluded 10 patients with acute promyelocytic leukemia. This study was approved by the institutional review board of the Ethics Committee and adds to the already described mutually exclusive mutations in germline RUNX1 results from other studies (8). Our study suggests that germline and somatic RUNX1 mutations in AML patients are mutually exclusive, as are several co-occurring pathogenic mutations that contribute to leukemogenesis. Our study adds to the already described mutually exclusive mutations in germline RUNX1 by identifying WT1, CHEK2, CCND3, and others. Similarly, in samples with somatic RUNX1 mutations, we found mutually exclusive mutations in CBL, JAK2, MLL, EZH2 and others, in addition to the previously described IDH1 (8). Further characterization of these results and analyses of additional samples using our whole-exome sequencing and our bioinformatics platform will help us better elucidate the molecular events underlying AML progression and help us establish novel prognostic/therapeutic markers aimed at early intervention in patients, or their family members, who carry RUNX1 mutations.

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

P195 Abstract withdrawn.

P196 MULTIPLE LEUKEMIC STEM CELL MARKER EXPRESSION IS ASSOCIATED WITH POOR PROGNOSIS IN DE NOVO ACUTE MYELOID LEUKEMIA

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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, which may be due to characteristics of residual disease. 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 (MyMRDTM), which allows characterization of the entire therapeutic AML biomarker repertoire and can inform us better stratification even among patients with intermediate-risk and favorable-risk karyotypes.

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.

Table 1. Univariate and multivariate analysis for OS.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate (OS)</th>
<th>Multivariate (OS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>1.00 (1.00-1.00)</td>
<td>1.00 (1.00-1.00)</td>
</tr>
<tr>
<td>NPM1 mutation</td>
<td>0.70 (0.51-0.94)</td>
<td>0.70 (0.51-0.94)</td>
</tr>
<tr>
<td>European Leukemia Net karyotype risk group</td>
<td>0.70 (0.51-0.94)</td>
<td>0.70 (0.51-0.94)</td>
</tr>
<tr>
<td>FLT3 mutation</td>
<td>0.70 (0.51-0.94)</td>
<td>0.70 (0.51-0.94)</td>
</tr>
</tbody>
</table>

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

P197 NEXT GENERATION SEQUENCING TARGETED PANEL FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA

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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, which may be due to characteristics of residual disease. 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 (MyMRDTM), which allows characterization of the entire therapeutic AML biomarker repertoire and can inform us better stratification even among patients with intermediate-risk and favorable-risk karyotypes.
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 myeloid malignancies.

**Methods:** Whole genome libraries, made from DNA extracted from cell lines and clinical samples, were hybridized with MyMRD probes targeting mutation hotspots in 23 genes associated with AML. In addition to single nucleotide variants (SNVs) and indels in 21 of these genes, 5 structural variant (SV) breakpoints in 3 genes were also targeted. Deep-sequenced 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, NPM1, and RUNX1.

**Results:** The linearity and limit of detection (LOD) of the MyMRD assay were assessed using data generated from controlled 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% – 2%). Overall, we established a LOD of 0.5% for >95% of the targeted sites in the assay with lower LODs for specific mutations of interest (e.g. 0.1% for a 30 bp FLT3 ITD and 0.2% for FLT3 p.D835V). In addition, using clinical samples, we found that all but one sample showed excellent concordance with the standard FLT3 CE assay for variants with VAFs above the CE detection threshold (5%). Sensitivity of the CE assay was nearly doubled when additionally evaluated with IVS FLT3 ITD MRD and NPM1 MRD amplicons which showed 100% concordance with the MyMRD panel assay for variants with VAFs above the MyMRD LOD.

**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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**P198**

**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. Recently, it has been 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 sets of mutations (53) (see Methods). However, we found that only 35% of the 3.5 amplicons were covered <500x, and more worryingly, we realised that at least one of those amplicons was shallowly (<100x) covered in 97% of the cases. Indeed both panels showed significantly lower average coverage levels of this gene compared to the panel as a whole (Figure 1). This might not be surprising, since CEBPA is not a CpG-rich region, and therefore its amplification needs tailored PCR conditions, hard to address in the multiplexed PCR step included in their library prep protocols. Therefore, both NGS approaches are prone to miss variants. In contrast, Sanger sequencing protocol (which includes optimized PCR conditions for correct amplification of the CEBPA gene) managed to cover the whole length of the gene. We were able to detect 26 variants affecting 20 AML cases through Sanger sequencing. Cases showing two variants were manually curated (through Chromos or IGV tools) to confirm if they affected different alleles. However, in 6 cases both mutations lay on different amplicons, which made not possible to univocally conclude if and how they were biallelic. These inconclusive cases were subjected to DMSO-Pfu-PCR in order to amplify the whole length of CEBPA coding region, followed by cloning. Colony sequencing showed independent clones harbouring different variants (i.e. bona fide biCEBPA mutations) in the majority of the cases, but crucially, not in all of them. This result highlights the need of implementing techniques able to accurately assess CEBPA biallelism, otherwise than plain calling of more than one variant.

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**P199**

**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 helps 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 until 2017 we investigated a total of 186 intensively 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 consolidation protocols.

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

**Summary/Conclusions:** Our data shows that MRD testing is routinely performed in \( \text{RUNX1-RUNX1T1} \) AML outside of clinical studies. Defining MRD levels by \( \text{RUNX1-RUNX1T1/ABL} \) ratios resulted in a better classifier for high and low risk patients than log fold change. However, despite CMR 16/90 (18%) patients relapsed with a maximum time from first achievement of CMR of 38.3 months. We conclude that 1) MRD monitoring could serve to guide BMT decisions in \( \text{RUNX1-RUNX1T1} \) AML outside of clinical studies. Defining MRD levels by \( \text{RUNX1-RUNX1T1/ABL} \) ratios resulted in a better classifier for high and low risk patients than log fold change. However, despite CMR 16/90 (18%) patients relapsed with a maximum time from first achievement of CMR of 38.3 months. We conclude that 1) MRD monitoring could serve to guide BMT decisions in \( \text{RUNX1-RUNX1T1} \) 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 MYELODYSPLASTIC SYNDROMES

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

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

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

Results: A total of 593 (42%) patients had MDS and 808 (56%) had AML. In a total of 984 (70%) patients, data on therapy with sufficient follow up and response evaluation was available, with 494 (35%) patients receiving therapy with hypomethylating agents (HMAs) and 373 (27%) with chemotherapy regimens. A total of 384 mutations in TP53, involving 208 unique mutations, were detected among 300 (21%) patients with AML (median age 67 years). The overall frequency of 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-occuring TP53 mutation was detected. Correlation between TP53 mutations and deletions (r=0.443, p<0.001) was observed with 172 (12%) patients having 1 TP53 abnormality, 169 (12%) having 2 TP53 abnormalities, and 19 (1%) having 3 abnormalities. 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: HR=2.81, CI 2.26-3.50, p<0.001). Increasing number of detectable mutations confer adverse prognosis irrespective of clinically available therapies. Correlation between number of TP53 abnormalities and their clinical relevance was observed with 172 (12%) patients having 1

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

Results: Two-hundred twenty-one patients were analyzed. Median age of the series was 54 years (range 18 to 72). 152 patients had a age 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 30 CEBPA biallelic wild type (CEBPα+/FLT3-ITD-). There were 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±18% 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-consolidation treatment, patients treated with HDAC only had an excellent prognosis, even better than those receiving an autologous or allelogeneic transplantation. One patient died in CR in the HDAC group, another in the autologous transplant group and 7 in the allo-HCT group (p<0001). In multivariate analysis of pretransplant characteristics, age up to 60 years and CEBPα+/FLT3-ITD- association 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 FMP have a good outcome. Best results are achieved in patients with CEBPα+/FLT3-ITD-, particularly if age is up to 60 years. In this subset, OS at 8 years is 96±7%, comparable to current results achieved in acute promyelocytic 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.

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

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

Aims: To evaluate the efficacy and safety of GMI-1271, a potent E-sel antagonist, in combination with induction chemotherapy in elderly untreated pts with AML.

Methods: Eligible pts had ECOG 0-2, WBC <40K/uL, no active CNS disease, and adequate renal and hepatic function. Prior treatment of MDS was allowed. GMI-1271 (10 mg/kg) was given 24 hrs prior, then every 12 hrs during and for 48 hrs post induction with infusional cytarabine and idarubicin (7+3). Two cycles of induction were allowed and responders could receive consolidation with GMI-1271 plus intermediate dose cytarabine. Dose-limiting toxicity (DLT), defined as myelosuppression in the absence of disease or related Grade 3 (Gr) non-hematologic toxicity beyond day 42, was assessed in the first 3 pts. Baseline E-selectin ligand expression on leukemic blasts in the bone marrow (CD45/SSC by flow) is reported.

Figure 1.
A Phase 2 Study of Glasdegib (PF-04449913) in Combination with Cytarabine and Daunorubicin in Untreated Patients with Acute Myeloid Leukemia or High-Risk Myelodysplastic Syndrome

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

Methods: Pts suitable for ICT (ECOG PS 0-1, creatinine ≤1.3 mg/dL, no severe sepsis, septic shock (1 pt each) and disease progression (2 pts). The observed steady-state plasma exposures for glasdegib were as expected at anti-neoplastic dose. Therefore, investigation of alternative agents for reactivation of PP2A is warranted.

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. Therefore, we conclude that this is a very promising option for AML, especially for treating leukemias characterized by SET dependence. 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.

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CM942 is a New Small Molecule that Targets PP2A Interaction and Inhibits Growth of Acute Myeloid Leukemia Cells

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Background: Acute myeloid leukemia (AML) is a heterogeneous malignant disorder of hematopoietic progenitor cells in which several genetic and epigenetic aberrations have been described. Nevertheless, outcome for most patients is poor, and it is necessary to develop more effective treatment strategies. Our group showed that the inactivation of the tumor suppressor PP2A is a recurrent event in AML, and that overexpression of SET, an endogenous inhibitor of PP2A, is a poor prognostic factor in this disease.

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

Methods: AML cell lines and 29 de novo AML samples were analyzed by treatment with FTY720 and CM942, MTS (viability), apoptosis, cell cycle and PP2A activity assays, and western blot.

Results: CM942 exhibited notable cytotoxicity on all human AML cell lines with SET overexpression (n=10). 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 effective than CM942 in primary samples of AML, fewer cytotoxic effects were observed after CM942 treatment in peripheral blood from healthy donors. Further experiments would be necessary to confirm the in vivo anti-tumor activity of CM942 in AML models. New compounds have been developed for the treatment of AML, although few have been translated into clinical practice; nevertheless, it is unlikely that any of these compounds, when used alone, will cure the disease, which is the ultimate goal of combinatorial 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.
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 (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.

CLONAL HETEROGENEITY IN LEUKEMIC STEM CELLS FROM PATIENTS WITH ACUTE MYELOID LEUKEMIA

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February 22, 2017

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TREATMENT OF PRACINOSTAT AND AZACITIDINE IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML): CORRELATION BETWEEN MUTATION CLEARANCE AND CLINICAL RESPONSE

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Background: In a phase 2 study of 50 elderly patients (>65 years) with AML who were not eligible for intensive chemotherapy, treatment with the investigational HDAC inhibitor pracinostat+azacitidine (AZA) was well tolerated and led to 42% complete remission (CR) and a median overall survival (OS) of 19.1 months (Blood 2016; 128:100). Responses were durable (median CR+CRi 17.2 months), blast clearance was rapid (median 8 weeks), and maximum clinical benefit required prolonged therapy (>6 months) in some patients.

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

Methods: 88 samples from 41 study patients were sequenced. Pre-treatment samples were available for analysis from all 41 patients, and a median of 3 longitudinal samples were analyzed from 19 patients between Cycle 2 and 9. Leukemia mutations were detected by SureSelect targeted capture exon sequencing (Agilent) of 295 genes that are recurrently mutated in hematologic malignancies (median coverage 507x [range: 111-777x]). Longitudinal mutation clearance was analyzed by tracking variant allele frequency (VAF). Informed consent was obtained from all patients.

Results: At baseline, 98 mutations in 28 genes were detected in 38 (93%) patients, with the most frequent being in SRSF2 (27%), DNMT3A (20%), IDH2 (17%), RUNX1 (17%), and TET2 (17%). The median number of mutations detected per patient was 2 (range: 0-6). Among the 33 patients with evaluable treatment response, CR was observed in 13 (39%) patients. The rate of CR was significantly higher in patients with mutations in NPM1 or in one of the DNA methylation pathway genes, while patients with TP53 mutation had a trend for poor CR (Table 1). The median follow up duration of the 41 patients was 23.8 months (95% CI: 20.4-27.1 months) with median OS of 18.1 months (95% CI: 10.1-26.1 months), patients with CEBPA mutation had a trend toward better OS, whereas patients with NFI mutation had significantly worse OS (Table). Considering mutations associated with AML oncogenesis (Lindsley RC, Blood 2015;125:1367-76), median OS was 17.7 months in 20 patients with mutations typically associated with secondary AML and 18.1 months in 18 patients with mutations typically associated with de novo AML. Among the 19 patients whose longitudinal specimens were analyzed, 10 achieved CR. Of those 10 patients, 9 (90%) had persistently detectable mutations in their bone marrow at the time of CR, however, in 7 of them, continued exposure to pracinostat+AZA lowered the VAF or cleared residual mutations. Mutations in genes associated with DNA methylation, RNA splicing, clonal hemopoiesis of indeterminate potential (CHIP), and receptor tyrosine kinase (RTK) pathways had poor clearance of mutation, while transcription factors or cohesin had better clearance with pracinostat+AZA treatment. In 2 patients, relapsed samples were sequenced and showed re-expansion of the founder clone.

Table 1.
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 ≤15x10⁹/L received AZA (75mg/m²x7 days [d]/28d cycle) or a CCR (IC [standard 7+3 regimen], low-dose cytarabine [20mg BID x 10d/28d cycle], or best supportive care). OS was assessed using Kaplan-Meier methods for pts with SD at 2-, 4-, and 6-month landmarks. SD was protocol-defined as the absence of an IWG-defined AML response and no progressive disease (PD), whether or not HI was attained. Pts with SD could have had an IWG-defined response or PD at any time other than at the specified landmarks. OS was also evaluated in pts with HI as their best response; attainment of HI must have begun on or before, and been sustained past, each landmark, and lasted for ≥56 consecutive days.

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 Txs arms, 1-year survival with AZA in pts with HI was 9.6%–33.3% greater than for CCR-treated pts with HI.

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

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

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Background: Fludarabine and clofarabine are purine nucleoside analogues with clinical activity in acute myeloid leukemia (AML). Aims: We designed a randomized phase II trial to evaluate the efficacy and safety of idarubicin and cytarabine with either clofarabine (CIA) or fludarabine (FIA) in adults with newly diagnosed AML. The primary objective was to compare the EFS rates of the two regimens. Methods: Adults with newly diagnosed AML deemed suitable for intensive chemotherapy were randomized using a Bayesian adaptive design to receive CIA or FIA. All patients (pts) received idarubicin 10 mg/m² IV on D1-3 and cytarabine 1 g/m² IV daily on D1-5. Clofarabine and fludarabine were given at doses of 15 mg/m² and 30 mg/m², respectively, IV daily on D1-5. Pts with FLT3-ITD mutations could receive concomitant sorafenib. Responding pts could receive up to 6 cycles of consolidation at attenuated doses. Outcomes were compared to a historical cohort of pts < 50 years of age who received idarubicin and cytarabine (IA) without a nucleoside analogue. Results: Between 8/2011 and 6/2016, 182 pts were enrolled (CIA, n = 106; FIA, n = 76; Table 1).

Table 1.

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<th>Parameter</th>
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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. Treatment arms were well-balanced after randomization. The median duration of follow-up was 27 months. The median duration of follow-up was 27 months.

P210

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


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Background: Approximately 20% to 30% of patients with acute myeloid leukemia (AML) have FLT3 mutations; these patients often experience rapid post-induction relapse, highlighting the need for therapies that provide an improved bridge to stem cell transplantation. CPX-351 is a liposomal formulation that delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin. CPX-351 demonstrated significantly prolonged overall survival (OS) versus cytarabine/daunorubicin (7 + 3) in a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, high-risk AML (Lancet, et al. ASCO 2016). A study of the ex vivo cytotoxicity of CPX-351 found that AML blasts with the FLT3-ITD mutation were 5-fold more sensitive to CPX-351 than those with wild type FLT3 (Gordon, et al. Leuk Res. 2017;53:39-49). Aims: The current analysis of the phase 3 trial therefore investigated outcomes in the subset of patients with FLT3 mutations.

Methods: Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m² cytarabine 100 mg/m²+daunorubicin 44 mg/m²) on Days 1, 3, and 5 (2nd induction: Days 1 and 3 only) or 7 + 3 (cytarabine 100 mg/m²/day x 7 days [2nd induction: x days]+daunorubicin 60 mg/m² on Days 1, 2, and 3 [2nd induction: Days 1 and 2 only]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 consolidation cycles. Results: Of the 274 patients who were assessed for FLT3 mutations and received study treatment, 22/138 (16%) patients in the CPX-351 arm and 20/136 (15%) patients in the 7 + 3 arm had baseline FLT3 mutations. AML subtypes in FLT3+ patients were: therapy-related AML (19%); AML after myelodysplastic syndrome/malignant mixed lineage disease (12%); and de novo AML with MDS karyotype (21%). In FLT3+ patients, median OS was longer with CPX-351 (10.25 months) versus 7 + 3 (4.55 months; hazard ratio=0.57 [95% CI: 0.24, 1.33]; P=0.093; see Figure 1), and the rate of CR+CRi was higher (45% vs 25%). A greater number of FLT3+ patients treated with CPX-351 were able to undergo stem cell transplantation (n=10 [22%] vs 44% [5%]; 4 patients were alive as of this analysis, after a median post-transplant follow up of 692 days [range: 96-769]) compared with 7 + 3 (n=2/20 [10%]; neither patient was still alive). The adverse event profile (reported during treatment or within 30 days of discontinuation) was consistent between CPX-351 in FLT3+ patients and 7 + 3. Common dose-limiting adverse events were consistent with the overall study population. Serious treatment-emergent adverse events (TEAEs) were experienced by 7 (32%) FLT3+ patients in the CPX-351 arm and 10 (50%) patients in the 7 + 3 arm; individual serious TEAEs in ≥2 patients included febrile neutropenia (n = 2 in each arm), respiratory failure (n = 1 and 2 in each arm), and cardiac failure (n = 2 with CPX-351), and cerebral hemorrhage (n = 2 with 7 + 3).

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

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](image)

**Figure 1.**

**P211**

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

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**Background:** Dose intensification and newer drug combinations during induction have led to high rates of complete remission (CR) in pts with newly diagnosed AML. However, disease relapse remains a major source of failure. With the exception of allogeneic (allo) stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk pts. Prior attempts to develop maintenance therapy using cytotoxic drugs in AML 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-consolidation remission.

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

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

**Results:** Eight pts have been treated, with a median age of 60 years (range, 49-71). 7 pts were in CR and 1 in CRi at the time of enrollment, 5 pts (63%) were in CR1, 2 pts (25%) were in CR2, and 1 pt (13%) in CR4 was inadvertently enrolled 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 cytotype, 1 (13%) adverse mutation 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 to hypothyroidism, treated successfully with steroids and thyroid hormone supplementation, who continues on treatment. 1 patient had grade 3 transaminase elevation which responded to dose interruption and who continues on treatment. 2 pts had grade 3 possible pneumonitis treated successfully with steroids and dose interruption – both were on treatment. Table 1 shows the patients with nivolumab by cycle. Summary/Conclusions: Nivo appears to be a feasible maintenance strategy in high-risk AML pts who are not candidates for SCT. The study continues to surpass the pre-specified expected rate of 6-month relapse-free survival of 22nd Congress of the European Hematology Association

**Table 1.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range)</th>
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<tbody>
<tr>
<td>Age</td>
<td>60 (56-73)</td>
</tr>
<tr>
<td>WBC [10^9/L]</td>
<td>3.8 (1.3-8)</td>
</tr>
<tr>
<td>Platelets [10^9/L]</td>
<td>125 (52-272)</td>
</tr>
<tr>
<td>DOX</td>
<td>451 (167-630)</td>
</tr>
<tr>
<td>Albinemia</td>
<td>3.9 (4.0-6.0)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.5 (11.9-14.5)</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.7 (0.5-0.8)</td>
</tr>
</tbody>
</table>

**P212**

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

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

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

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

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

Summary/Conclusions: Higher expression of KIAA0125 in AML patients was correlated with mutations of RUNX1, 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

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

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

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

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

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

P214

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

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

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

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

Methods: The present retrospective cohort study included adult patients with untreated AML consecutively diagnosed between 2004 and 2014 in the Hematology Unit of the University-Hospital Città della Salute e della Scienza of Torino, Italy. The study was approved by the Ethical Committee and was registered at www.clinicaltrials.gov as NCT02714790. Among 255 enrolled patients, MRD was investigated in those in first complete remission (CR) with an available at diagnosis and at two further time-points: after induction (n=117) and prior allo-HCT (n=65). Patients with baseline WT1 >250 copies were excluded. All patients underwent intensive induction chemotherapy with curative intent and subsequent consolidation chemotherapy according to the AML risk assessment (autologous peripheral stem cell transplantation for low risk and allo-HCT for intermediate and high risk patients).

Effect of post induction WT1 expression on disease-free survival (DFS) and overall survival (OS) and of pre allo-HCT WT1 expression on the cumulative incidence of relapse (CIR) were investigated.

Results: Baseline WT1 expression were not found significantly associated with demographic, clinical and disease biological features at diagnosis. Baseline BM WT1 expression lacked even to show an association with response to induction chemotherapy (OR 1.16; 95% CI 0.90-1.50, p=0.24)

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 2.1; 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 greater impact. Published evidences available so far supported these suggestions, but mainly due to methodological issues, the role of WT1 is still a matter of debate. Perspective randomized studies are required to confirm these results.

P215

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

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Background: Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 enzymes. Preclinical studies showed that exposing myeloblasts from patients (pts) with acute myeloid leukemia (AML) to enasidenib ex vivo resulted in differentiation of leukemic marrow blasts into mature, functionally normal neutrophils (Ven et al, 2017). Enasidenib can result in IDH-inhibitor-associated differentiation syndrome (IDH-DS) in treated pts, with manifestations akin to retinoic acid syndrome seen during therapy of acute promyelocytic leukemia.

Aims: To characterize the prevalence, characteristics, and course of IDH-DS in relapsed or refractory (R/R) AML receiving enasidenib 100 mg daily in a phase 1 dose-escalation and expansion study (NCT01915498). This dose is currently under study in a multicenter, randomized, phase 3 trial comparing enasidenib with conventional care regimens in R/R AML pts (NCT02577406).
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 mIDH2 AML 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 examination, standard biological parameters, bone marrow biopsy, CT scan at baseline, CT response evaluation and outcome).

Results: We identify 65 IVLBCL patients treated in 23 LYSA centers during the studied period. Median age was 67.8 years (range 22-91). In note, two patients presented with IPI score >3 (67%) and all patients had a stage IV disease. As expected in Western patients, cutaneous and CNS involvement were highly frequent, respectively 33% and 39%. But interestingly, hemophagocytic syndrome were observed in nearly half of the patients (41%), while it was mainly described in Asian series. Despite classically delayed diagnosis in IVL- BCL, only 2 cases were confirmed post-mortem and almost all alive patients at diagnosis (n=58) were treated with rituximab-containing chemotherapy regimens (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 29.4 months and median overall survival 63.8 months (Figure 1). Pathological features (including cell of origin characterization, C-MYC expression, adhesion protein expression level) investigation is ongoing and will be presented at the time of the meeting.

Figure 1.
P218
OUTCOME OF ELDERLY DLBCL PATIENTS (≥80 YEARS) TREATED WITH ANTHRACYCLINE BASED CHEMOTHERAPY. R-CHOP DOSE REDUCTION IS NOT NECESSARY FOR EVERYBODY
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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. (30.9%) 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 or intermediate high IPI 49.1%, with bulky disease (>10 cm) 17.0%, with lower albumin 27.7%, with Charlson Comorbidity Score (CCS) ≥4 25%. According to treatment choice of physician (intent to treat), pts. could be divided into 3 groups R-CHOP (CH) (cyclophosphamide – CF 750 mg/m2, adriamycin – A – 50 mg/m2) or R-MiniCHOP (miniCH) (CF 400 mg/m2, A 25 mg/m2, Peyrade 2011) or modified R-CHOP (modiCH) (CF 750 mg/m2 and A 25 mg/m2) or any other dose between CHOP and miniCHOP. There were 21 pts (18.8%) treated with CH, 38 (33.3%) with miniCH and 53 (47.3%) with modiCH.

There were no significant differences between the subgroups, except higher proportion of bulk in modiCH vs miniCH and CH (35% vs 12.9% vs 7.7% resp.; p 0.04) and cardiac comorbidity (60.5% vs 33.3% vs 30.2% resp.; p 0.02). Six and more cycles were administered in 71.4%, 63.1% and 58.5% pts. in CH, miniCH and modiCH resp. Following proportion of pts. received ≥80 (≥50%) of original CHOP dose. For cyclophosphamide it was 66.7% (81%), 0% (50%) and 62.2% (79.2%) resp. and for A it was 57.1% (76.1%), 2.6% (15.8%) and 13.2% (49%) resp. for CH, miniCH and modiCH resp. There were observed 11 treatment related deaths (6 cardiac toxicity and infection), 5 in miniCH and 6 in modiCH groups. The overall response rate was 76.8% with 59.8% CR/CRu. Median PFS and OS were 2.8y and 4.6y vs 3.5y and 4.0y in the last period. The age-specific incidence rates (1989-1994, 1995-2000, 2001-2007 and 2008-2014) and 3 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 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.
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 age 70 or below. This is largely explained by the increased use of intensive therapy over time. Although the use of CT alone generally increased among pts >70 years, their survival is still poor. Therefore, there is an urgent need to design specific trials for elderly PCNSL pts to improve their survival.

P220

CLINICAL CHARACTERISTICS AND LONG-TERM RESULTS OF TREATMENT OF DIFFUSE LARGE HEPATITIS C-ASSOCIATED NON-HODGKIN LYMPHOMA (DLBCL+C)
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Background: In the WHO classification (2008), hepatitis C virus distinguish as one of the etiological factors of multistage elopogenesis DLBCL.

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

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

Results: Patient’s age ranged from 21 to 76 years (median was 47 years) in DLBCL+C; ranged from 23 to 81 years (median 61) in DLBCL-C (p=0.02). The male: female ratio was 1: 1.3 in patients with DLBCL+C: 1: 1.7 in the group DLBCL – C. Stage I and II were in 11% patients with DLBCL+C, and 48% patients with DLBCL - C; III and stage IV were detected in 89% patients with DLBCL+C and 52% of DLBCL-C (p=0.00002). Extranodal lesions detected in 72% in DLBCL+C and in 26% in C DLBCL-C (p=0.006). In comparable groups localization of extranodal lesions was: spleen (52% to 23%), bone marrow involvement (43% and 27%), liver (18% and 1%), lesions was: spleen (52% to 23%), bone marrow involvement (43% and 27%), liver (18% and 1%), GCB / non-GCB histological variants ratio was 55% / 45% in DLBCL+C; 36% / 64% in DLBCL-C group / ratio GCB / (p=0.001). Hepatitis C virus RNA blood were identified by ELISA. All patients received chemotherapy according to the scheme CHOP / R-CHOP. The frequency of complete remission was 60% in the group of patients with DLBCL+C and 63% of DLBCL-C. Median overall survival (OS) was 46 months in group DLBCL+C and 71 months in DLBCL-C (p=0.0003).

Median progression-free survival (PFS) was 28 months in DLBCL+C 47 months in the control group (p=0.0002). According to the immunohistochemical variant of DLBCL: GCB DLBCL-C and 42 months in group non-GCB. In DLBCL+C 62 months in non-GCB DLBCL-C (p=0.0002). Median PFS was 36 and 47 months in comparable group. Median OS was 18 months in non-GCB DLBCL+C and 70 months in non-GCB DLBCL-C (p=0.00001). Median PFS groups was 13 and 42 months, respectively. 58 patients received antiviral therapy after chemotherapy. Median OS was 63 months in GCB DLBCL+C with antiviral therapy and 28 months in GCB DLBCL-C without antiviral therapy (p=0.00002). Median PFS was 46 and 20 months, respectively. Median OS was 22 months in non-GCB DLBCL+C with antiviral therapy and 17 months in non-GCB DLBCL-C without antiviral therapy. Median PFS in the group was 11 and 15 months, respectively.

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

P221

MAGNETIC RESONANCE IMAGING FOR EARLY DETECTION OF ANTHRACYCLINE CARDIOTOXICITY IN MALIGNANT LYMPHOMA
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Background: Doxorubicin is a cornerstone of curative lymphoma treatment. However, doxorubicin therapy is limited by cardiac side effects including high-mortality heart failure (HF). Signs of cardiotoxicity often appear too late to avoid irreparable myocardial damage.

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

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

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

Figure 1.

Summary/Conclusions: Our preliminary show that cardiac MR can be used for detection of declining LV function 1 year after doxorubicin exposure. It appears that cardiac MR may also provide information on acute functional changes in LVEDV and SV. We hope that our 82 Rb PET and 123I MIBG data will provide additional early signs of doxorubicin cardiotoxicity that can be used to predict subsequent development of HF.
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 date of last follow-up longer than 12 months. All patients in CR after frontline therapy were followed-up with clinical visits (symptom assessment, physical examination, and blood tests) every 1 to 6 months. Surveillance CT covering the neck, chest, or abdomen were performed every 3 or 6 months or when clinically indicated in the first 2 years, and then every 6 or 12 months or when clinically indicated thereafter. The decisions regarding the surveillance strategy (clinical visit with appropriate blood chemistry with or without surveillance CT) were at the discretion of the treating physicians.

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 by surveillance CT did not improve the 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 treated with GLIDE (gemcitabine, L-asparaginase, ifosfamide, dexamethasone and etoposide) chemotherapy reported complete response (CR) rate and 3-year overall survival (OS) of these patients were 57.1% and 56% respectively. We assumed autologous stem cell transplantation may further improve the prognosis of these patients.

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

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

Results: Fifty-seven patients had finished planned treatment with 1 withdraw of informed consent after cycle 1, and 2 death of sepsis during cycle 1 and cycle 2 respectively. Twenty-one patients underwent ASCT. The ORR was 81.4% and the CR was 69.5% with early CR (CR after 2 cycles) of 57.6%. Estimated 5-year OS and PFS rates of the whole cohort and patients underwent ASCT were 68.7%, 54.0%, 79.6% and 85.2% respectively. Univariate analysis revealed that ECOG ≤1, IPI ≤2, early CR and ASCT were associated with less relapse and death. Multivariate analysis showed ECOG ≤2 was an independent risk factor for disease progression (HR=4.321, 95% CI 1.127–16.572, P=0.033) and death (HR=46.254, 2.150–993.190, P=0.014) and ASCT was associated with better PFS (HR=0.058, 95% CI 0.007–0.495, P=0.009) and OS (HR=0.019, 95% CI 0.001–0.596, P=0.024). Figure 1 highlights the OS and PFS of whole cohort (A) and ASCT patients (B). Myelosuppression was the most common adverse reaction (AE). The incidences of level 4 neutropenia, thrombocytopenia and anemia were 46.6%, 28.6% and 5.3% respectively. The most common non-hematologic AE was fever with neutropenia (36.5% of total cycles), while others were mild and manageable.

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

P226
IDENTIFICATION OF A NOVEL GERMLINE MECOM / EV1 VARIANT THAT RUNS IN A PEDIGREE WITH RADIOULNAR SYNOSTOSIS AND AMEGAKARYOCYTIC THROMBOCYTOPENIA AND PREDISPOSES TO ADULT ONSET MYELOID MALIGNANCY

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

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

Methods: Whole exome sequencing was performed in three affected individuals using a Nextera Rapid Capture kit and a NextSeq 500 instrument (Illumina, Munich, Germany). Identified sequence variants were filtered for those that are reported to be functionally relevant in databases (ESP6500, ExAC). In three simplex patients, de novo missense variants in MECOM have recently been reported as an alternative cause in individuals with RUSAT. MECOM, identified as a common ecotropic viral integration site 1 (EV1) in murine myeloid leukemia, is known as a key transcriptional regulator in hematopoiesis and is frequently involved in sporadic myeloid leukemia.

Results: 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].

P227
LOSS OF THE HOMOLOGOUS RECOMBINATION GENE RAD51 LEADS TO FANCONI ANEMIA-LIKE SYMPTOMS IN ZEBRAFISH

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Background: Fanconi anemia (FA) is a hereditary DNA repair disorder characterized by various congenital abnormalities, progressive bone marrow failure and cancer predisposition. RAD51 has recently been designated as a Fanconi anemia (FA) gene, following the discovery of two patients carrying dominant negative mutations. RAD51 is an indispensable homologous recombination protein, necessary for strand invasion and crossing over. It has been extensively studied in prokarocytes 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. As FA is a serious disease, we aim to utilize the powerful genetics and translucency of zebrafish to dissect the role of rad51 in hematopoiesis and to explore the molecular basis of Fanconi anemia pathogenesis.

Methods: Zebrafish carrying homoygous loss of function mutations in rad51

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generated by ENU mutagenesis were characterized in terms of their hematopoietic and non-hematopoietic phenotypes during embryonic development and adulthood.

Results: The rad51f mutant fish developed key features of FA, including hypocellular 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 10 µm respectively.

Summary/Conclusions: We demonstrate that zebrafish lacking functional rad51f are viable and develop symptoms resembling FA. These findings strengthen the assignment of RAD51f as a Fanconi gene and provide more evidence for the notion that aberrant p53 signaling during embryogenesis leads to the hematological defects seen during later stages of life in FA patients. Further research on this novel zebrafish FA model will lead to a deeper understanding of the molecular basis of bone marrow failure in FA and the cellular role of the RAD51 protein.

P228
A NOVEL TELOMERASE RNA COMPONENT VARIANT IN A FAMILY WITH MACROCYTOSIS AND MILD VARIABLE CYTOPENIAS
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Background: Telomerase RNA component (TERC), encoded by the TERC gene, is an essential component of telomerase, a polymerase that adds the telomeric repeat to the 3’ lagging strand of DNA during cell replication. TERC variants have been causally associated with several haematological disorders, including autosomal dominant dyskeratosis congenita (DKC), aplastic anaemia, myelodysplastic syndrome and acute leukaemia, sometimes accompanied by non-haematological phenotypes. Here we report a likely pathogenic TERC variant associated with a haematological phenotype that predominantly affects the red cell lineage.

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

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

Results: The index case AM (I.1) was a female who presented at age 56 with lifelong macrocytosis and previous mild neutropenia (Table 1). The younger daughter (age 30) TW (II.1), had isolated lifelong macrocytosis and previous mild neutropenia (Table 1). The younger daughter (age 27) BM (II.2) had macrocytic anaemia, thrombocytopenia (Table 1) and a recent pregnancy complicated by worsening thrombocytopenia, pre-eclampsia, placental dysfunction, liver dysfunction and foetal loss. Following delivery her liver function slowly returned to normal and a fibroscan was within normal limits. All three pedigree members with macrocytosis had a n.181A>C substitution in TERC (relative to transcript ENST00000602385.1), within the pseudoknot domain. Residue n.181 is highly conserved across mammalian species. This variant is absent from the gnomAD database of more than 230,000 TERC alleles, and the HGMD databases. The variant is within a TERC region in which previously reported variants have been associated with haematological phenotypes. In order to determine the pathogenicity of this variant, telomere lengths were assessed and found to be short in both Case I.1 and II.2 (Table 1). There were no other likely pathogenic variants in the Oxford Red Cell Panel genes. Together, these observations suggest that the n.181A>C substitution is causally associated with the macrocytosis phenotype.

Table 1.

<table>
<thead>
<tr>
<th>Parameter (normal range)</th>
<th>II.1 (case)</th>
<th>I.1 (mother)</th>
<th>BM (daughter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>94.1</td>
<td>87.0±4.6</td>
<td>89.0±9.2</td>
</tr>
<tr>
<td>Mean cell volume (fl)</td>
<td>111.6</td>
<td>95.8±3.9</td>
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<tr>
<td>Mean cell haemoglobin (g/dL)</td>
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<td>38.1±5.0</td>
<td>39.2±1.0</td>
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<tr>
<td>Total white cell count (×10^9/L)</td>
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<td>4.0±0.7</td>
<td>6.3±0.5</td>
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<tr>
<td>Neutrophils (×10^9/L)</td>
<td>1.8±0.7</td>
<td>1.7±0.6</td>
<td>2.0±0.6</td>
</tr>
<tr>
<td>Lymphocytes (×10^9/L)</td>
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<td>2.0±0.6</td>
<td>1.5±0.4</td>
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<tr>
<td>Platelets (×10^9/L)</td>
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<td>110.0</td>
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<tr>
<td>Serum ferritin (µg/L)</td>
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<td>35.0±20.0</td>
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<tr>
<td>Total protein (g/L)</td>
<td>71.0</td>
<td>54.0±12.0</td>
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<td>21.5%</td>
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</tr>
</tbody>
</table>

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

This report demonstrates a likely causal association between a newly identified TERC variant, short telomere length and a relatively mild haematological phenotype that is largely restricted to red cells. This 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: TELOMERE LENGTH SCREENING TRIGGERED BY CLINICAL SUSPICION performance of the eculizumab can be in part explained by its action on EVs.

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 HSCs. The generation of X-DC-like HSCs will facilitate the understanding of the molecular basis of the HSC defects characteristic of X-DC and contribute to the development of new experimental therapies for the treatment of the BMF of X-DC patients.

STUDY OF EXTRACELLULAR VESICLES ROLES IN THE PATHOPHYSIOLOGY OF THROMBOSIS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA PATIENTS DURING ECUKLIZUMAB TREATMENT: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY A. Wannez1,2,*, B. Devalet2, C. Boury1, B. Bihin3, J.-M. Dogné1, F. Mullier2

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

Aims: The general purpose of this project is a better understanding about the role of EVs in thrombosis in the context of PNH patients under eculizumab. We also assessed the procoagulant activity in PNH patients treated with eculizumab, 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 Helsinki Helsinki and approved by the local Ethic 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, after 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 pelletted 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 with 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-thrombotic performance of the eculizumab can be in part explained by its action on EVs.

TELOMERE LENGTH SCREENING TRIGGERED BY CLINICAL SUSPICION FOR CLASSICAL AND/OR CRYPTO DSYKEROSITIS CONGENITA – PROSPECTIVE RESULTS FROM THE AACHEN TELOMEROPTHY REGISTRY

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Background: Classical Dyskeratosis Congenita (DKC) is a multisystem disorder caused by defective telomere maintenance, mostly due to mutations in genes related to functional activity of telomerase or accessibility of the telomeres themselves. Clinical characteristics are mucocutaneous abnormalities, bone marrow failure, an increased predisposition to cancer and other variable features. While classical DKC is typically diagnosed in childhood or adolescence, the incidence of cryptic DKC variants typically presenting with a clinically more heterogeneous picture is increasing. The diagnostic system of DKC in patients, in which the age of onset is unknown. Accelerated shortening of Telomere length (TL) in peripheral blood leucocytes represents the functional read-out of altered telomere maintenance and thus allows for a screening of patients with suspected DKC. On the basis of TL measured below the 1% percentile of age adjusted healthy controls, next generation-sequencing (NGS) analysis for identifying mutations was triggered.

Aims: In this study, we report the first results of such a clinical routine screening for telomeropathies carried out within the Aachen Telomeropathy Registry (ATR).

Methods: 184 patients from 52 participating centers (80% academic centers) within Germany, Austria and Switzerland were screened for premature telomere shortening and included with informed consent into the ATR since November 2014. Inclusion criteria and reason for screening was either the clinical suspicion of the treating physician for a telomere maintenance disorder and/or the recommendations of the German Society of Hematology and Oncology (DGHM) published via Onkopedia. TL analysis of peripheral blood granulocytes and lymphocytes was carried out using combined fluorescence in situ hybridization and flow cytometry (flow-FISH). Mutations in genes suspected to cause telomeropathies (i.e. TERT, TERC, DKC1, NOP10, NHP2, USC1, CTCL, RT1L1, TIN2, TACB1) were analyzed by NGS using customized primer panels and amplicon-based sequencing on a MiSeq sequencer (Illumina) in all patients with TL in lymphocytes below the 1% percentile of healthy controls.

Results: Underlying initial diagnosis by the treating physician with typical telomere screening were aplastic anemia (AA, n=72, 39% of cases), unexplained cytopoiesis disturbance (UC, n=16, 8.6%), and lymphocytes (n=21, 11%), malignancies (n=11, 5.9%), family members (FM) of known DKC patients (FM-DKC, n=17, 9%), atypical squamous cell cancer of the head-and-neck (SCCHN, n=10, 6%), paroxysmal nocturnal hemoglobinuria (PNH, n=9, 5%), acute myeloid leukemia (AML, n=5, 3%) as well as other disorders (e.g. lung fibrosis, Diamond-Blackfan-Anemia, thrombocytopenia, etc.). Median age of patients with mutations was 45.0 y (range 21 to 68 y). TL screening revealed 20% (38/184) patients with lymphocyte TL and 16% (30/184) of patients with granulocyte TL below the 1% percentile. NGS screening identified typical mutations associated with altered telomere maintenance in 15 out of 38 patients (40%) representing 8.2% of the total patient population. Median age of patients with mutations was 45.0 y (range 21 to 68 y). Mutations were detected in RTEL1 (n=3), TIN2 (n=3), DKC1 (n=3). Mutations were observed in 5% of all AA, 12% of all UC, 50% of all AML-DKC, 13% of all SCCHN, 20% of all screened AML patients.

Summary/Conclusions: We provide the first analysis of a routine TL screening for telomeropathies in a large cohort of patients under investigated telomere screening. Under the age of 40: Mortality has not been reduced in 2010-2015.

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Background: Mortality following HSCT in SAA pts over the age of 40 is reported to be in the order of 50%, without taking into account long term sequelae such as chronic GvHD, known to be more frequent in older patients. This has prompted international guidelines to recommend first line immunosuppressive therapy above 40 years of age. The question is whether this is still true in 2017.

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

Methods: We used the WPSA-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 (54% vs 42%, p<0.01).

Results: The overall survival 5 year survival of pts grafted in 2001-2009 was 57%, compared with 55% for pts grafted 2010-2015 (p=0.7). In multivariate analysis, including the interval diagnosis transplant, patient's age, donor type, stem cell source and conditioning regimen, the lack of improved survival in 2010-2015 was confirmed (p=0.3). A very strong age effect was shown both in univariate and multivariate analysis: survival of pts aged 40-50 years, 51-60 years and >61 years, was respectively 64%, 54%, 41% (p<0.0001) and this was confirmed in multivariate analysis. The conditioning regimen, also proved to be a significant predictor, with improved survival for ALT transplants receiving FLU containing regimens (56% vs 46%, p<0.001). In general pts receiving either CY200 or a FLU containing regimen, did significantly better than pts receiving other preparative regimens (58% vs 50%, p=0.02). The use of a sibling donor (SIB) did not prove to predict survival in multivariate analysis. Pts receiving Campath in the conditioning, did significantly better than pts not receiving Campath (65% vs 54% p<0.01); similarly survival of patients with ATG was superior 59% vs 41% compared to patients not receiving ATG (p<0.01). When pts receiving either Campath or ATG (n=564) were compared to patients not receiving either (n=161), the difference in survival was 61% vs 41% (p<0.0001), and this was significant also in multivariate analysis. Combined primary and secondary graft failure was reduced from 16% to 12% in the two time periods (p=0.02), acute GvHD grade II-IV was reduced from 15% to 11% (p=0.01) and chronic GvHD was also reduced from 32% to 26% (p=0.04). 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).

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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CLINICAL AND GENETIC DIVERSITY IN DIAMOND-BLACKFAN ANAEMIA: AN UPDATE FROM THE UNITED KINGDOM

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Background: Diamond-Blackfan anaemia (DBA) is an inherited bone marrow failure syndrome (BMFS) caused by mono-allelic, loss-of-function mutations in ribosomal protein (RP) genes. DBA is rare and has a wide spectrum of clinical manifestations, hence the utility of patient registries.

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

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

Results: The 103 patients with DBA were born in a 48-year period (1967-2015), i.e., an incidence of 3 per million live births. Demographic and clinical characteristics are shown in Table 1. NGS analysis of 80 RP genes plus GATA-1 identified pathogenic mutations in 71% of cases and 7 putative novel mutations, currently undergoing validation. To date, mutation screening of both parents has been performed in 32 families with DBA. Twenty-five mutations are sporadic while 7 are autosomal dominant; in 3 of the latter, the parent is a silent ‘carrier’ without anaemia. In one case of an affected child, the causative mutation was detected in the peripheral blood of both parents but was present in 722 embryos generated for in vitro fertilisation, suggesting germline mosaicism. 80.5% of cases in our cohort presented within the first year of life. For the first time we report a high rate of perinatal problems in DBA. Prematurity +/- intrauterine growth restriction (IUGR) occurred in 31/87 (35.6%) of evaluable patients. Specific aberrant abnormalities included: hydrops fetalis (3/87), prematurity (22/87) and IUGR (16/87). In addition to congenital anomalies classically associated with DBA, we identified abnormalities of the spine and axial skeleton in 9.2% of patients. These did not correlate with a particular genotype. Our cohort exhibited multiple comorbidities, including some not previously reported to be associated with DBA: hematoma (10.7%), neuropsychiatric (17.4%) and gastrointestinal (GI) disorders (25.7%). These complications were not associated with particular treatment regimens. In terms of the natural history of DBA, a lower proportion of our patients (22%) than previously reported in the literature (40%) were able to maintain a normal Hb on long-term steroids. Three patients failed conventional therapy to date. In total there were 4 individuals with brainstem atrophy (BAS), BCC and cervical intraparietal neoplasia) in 4 different patients. The lower incidence in our cohort compared with that reported by the North American DBA registry may be explained by differences in the median ages of the 2 cohorts (12½ vs 18½, respectively) and the shorter follow-up of our patients.

Table 1.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Number of Cases (% of Total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prematurity/low birth</td>
<td>31/87 (35.6%)</td>
</tr>
<tr>
<td>IUGR</td>
<td>22/87 (25.7%)</td>
</tr>
<tr>
<td>Spine anomalies</td>
<td>8/87 (9.2%)</td>
</tr>
<tr>
<td>Axial skeleton anomalies</td>
<td>9/87 (10.7%)</td>
</tr>
<tr>
<td>Hematoma</td>
<td>6/87 (7.0%)</td>
</tr>
<tr>
<td>Neuropsychiatric disorders</td>
<td>15/87 (17.4%)</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td>22/87 (25.7%)</td>
</tr>
</tbody>
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Summary/Conclusions: This retrospective analysis of the UK’s DBA cohort confirmed several findings from other registries but also revealed novel features, including a high prevalence of i) premature birth and neonatal complications ii) abnormalities of the axial skeleton and iii) neuropsychiatric disorders. Prospective longitudinal studies are warranted to better characterise these co-morbidities and confirm whether they are intrinsic to DBA or arise as complications of treatment. Above all, the observed clinical heterogeneity in our cohort highlights the need for novel therapies that target the multisystem manifestations of DBA, not just the anaemia.
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 2005-2016, 88 patients have been studied for single-lineage (25) or multilineage (63) MF. 48 (64%) were classified as having an underlying PID. Table 1 shows clinical characteristics and mutations of patients with PIDs.

Table 1.

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

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

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Background: Paroxysmal nocturnal haemoglobinuria (PNH) leads to episodic haemolysis secondary to an acquired deficiency of PIGA anchoring 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 anti-body 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.
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GERMLINE RARE VARIANT ASSOCIATION ANALYSIS IN CHRONIC LYMPHOCYTIC LEUKAEMIA

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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 IGCCCG. 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 variants (MAF <0.01), and sought to identify these through an exome-wide association study comparing rare germline variants between CLL patients and controls.

Results: We searched for patients with rare coding variants in the majority of affected cases and controls; and (iii) analyzing only ethnically matched, unrelated samples over DNA sites with sequencing coverage sufficient to achieve high-confidence genotype calls. This quality control resulted in 8,920 controls available for the association analysis. We further controlled for residual population stratification by correcting for three principal components. 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 (TREs) and provides a sensitive, quantitative and directional map of transcriptionally-engaged RNA polymerases. The algorithm, discriminative regulatory-element detection from PRO/PRO-seq (dREG), is then used to predict the presence of TRES from raw PRO-seq data, allowing for identification of functional elements in the vicinity of SNPs and quantification of their allele-specific effects on enhancer activity and gene transcription.

Results: Our gene expression analysis demonstrated 6 significant SNP-gene associations: rs674313 (6p21.3) with HLA-DQA1 (p <0.0001), rs8720701 (6p25.3) with HLA-DQA1 (p=0.01), rs7771784 (15q23; proxy for rs1767508) with TP53 (p=0.09), rs783545 (15q23.2) with COX4NB/EMC8 (p<0.0001) and rs482232 (18q13.32; proxy for rs11083846) with FRK (p <0.0001). Two associations were successfully validated in a completely independent gene expression replication analysis (n=54): rs674313 with HLA-DQA1 (p <0.0001) and rs7771784 with TP53 (p=0.0116). To annotate candidate regulatory elements, we evaluated transcription level at or near all six significant functional elements in the initial gene expression analysis in a cohort of 12 CLL samples. Transcription level at or near 3 SNPs (rs674313, rs7771784, 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 B and BIALLIC sample sets (p <0.0001).

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 that influence nearby target genes.

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 rs4771184 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 in tumor samples using 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 (TREs) and provides a sensitive, quantitative and directional map of transcriptionally-engaged RNA polymerases. The algorithm, discriminative regulatory-element detection from PRO/PRO-seq (dREG), is then used to predict the presence of TRES from raw PRO-seq data, allowing for identification of functional elements in the vicinity of SNPs and quantification of their allele-specific effects on enhancer activity and gene transcription.

Results: Our gene expression analysis demonstrated 6 significant SNP-gene associations: rs674313 (6p21.3) with HLA-DQA1 (p <0.0001), rs8720701 (6p25.3) with HLA-DQA1 (p=0.01), rs7771784 (15q23; proxy for rs1767508) with TP53 (p=0.09), rs783545 (15q23.2) with COX4NB/EMC8 (p<0.0001) and rs482232 (18q13.32; proxy for rs11083846) with FRK (p <0.0001). Two associations were successfully validated in a completely independent gene expression replication analysis (n=54): rs674313 with HLA-DQA1 (p <0.0001) and rs7771784 with TP53 (p=0.0116). To annotate candidate regulatory elements, we evaluated transcription level at or near all six significant functional elements in the initial gene expression analysis in a cohort of 12 CLL samples. Transcription level at or near 3 SNPs (rs674313, rs7771784, 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 B and BIALLIC sample sets (p <0.0001).

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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BIALLIC TP53 GENE MUTATIONS DUE TO COPY-NEUTRAL LOSS OF HETEROZYGOSITY AND MONOA LLEIC MUTATIONS IN ABSENCE OF 17P DELETION OCCUR IN CLL WITH COMPARABLE FREQUENCY

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Background: TP53 gene defects represent an adverse prognostic marker in chronic lymphocytic leukemia (CLL). In the majority of affected cases, TP53 is inactivated on both alleles due to the concurrent mutation and 17p deletion (del(17p)). However, in about one third of cases, only TP53 mutation (TP53mut) without deletion is detected. It was reported that in some of these patients, copy-neutral loss of heterozygosity (cn-LOH), also leading to biallelic TP53 defect, might be present; however the frequency of such event has not been thoroughly investigated.

Aims: We aimed to perform a detailed analysis of the second TP53 allele status in cases with a TP53 mutation in the absence of del(17p), and to assess genomic and clinical outcome in these patients.

Methods: We searched for patients with TP53mut in absence of del(17p) within the cohort of 200 CLL patients positive for TP53mut as determined using FASAY (Functional Analysis of Separated Alleles in Yeast) coupled to direct sequencing;
17p13 deletions were assessed by FISH (MetaSystems). More than a half of the cohort (57%) was also examined using ultra-deep NGS for TP53 exons 2-11. Genome-wide analysis was performed on CytoScanHD arrays (Affymetrix) and correlated to conventional cytogenetics (CpGILIL-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 unobserved by FISH was newly revealed. Thus, the truly monoallelic mutations were confirmed. We applied various methods, where by no cytogenetic abnormality in 17p locus was observed (median TP53 VAF 43.5%, range 10.5–51.3%). Applying a VAF cut-off of 55% indicating fully expanded heterozygous mutation (taking into account the potential unequal representation of forward and reverse strands in NGS data), 7/29 (24%) cases below the cut-off still harbored 17p cn-LOH. These results show that it is not possible to use an arbitrary VAF cut-off (>50%) to identify biallelic mutations due to cn-LOH. When we compared genomic complexity of leukemic clones with monoallelic vs biallelic TP53mut as determined by the CytoScan array, the latter group exhibited significantly more genomic abnormalities (p<0.0388) and also preference for different recurrent chromosomal abnormalities (p<0.0001: 17p locus excluded from this analysis). However, there was no significant difference in overall survival between the groups (p=0.5856).

**Summary/Conclusions:** cn-LOH in 17p locus is present in approximately half of the patients with single dominant TP53mut and results in biallelic TP53 gene inactivation despite the absence of del(17p). Truly monoallelic TP53 gene mutations with an intact second allele occur in CLL in comparable frequency. Although 17p cn-LOH is associated with increased genomic instability, it does not have a worse impact on clinical outcome than truly monoallelic TP53mut. Supported by the projects AZV-MZCR 15-31834A, 15-30015A, 15-29733A, the EU Horizon2020 project No. 692928, and MEYS GITEC2020 LQ1601.

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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:** B-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 on long arm 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 profile. Recently clinical trials with tyrosine kinase inhibitors such as ibrutinib and idelalisib have demonstrated a high rate of cytogenetic improvement following chemotherapy, particularly emergence of del(17p). Additionally, pts with del(17p) or TP53 mutations frequently develop additional copy number alterations (CNAs) following chemotherapy.

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

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

**Results:** In total, 37 of 336 pts (11%) progressed during ibrutinib-based therapy. Of these pts, 15 had FISH analysis both pre-treatment and at progression: pre-treatment, 10 had del(17p), 1 had del(11q) and 4 had isolated del(13q). All 4 of the pts with isolated del(13q) pre-treatment had new sub-clonal TP53 abnormalities detected at progression: two pts had del(17p), 1 had del(11q) and 4 had isolated del(13q). In the absence of disease progression, the only CCE detected was emergence of small sub-clones with biallelic del(13q) in two patients who initially had monoallelic del(13q). Notably, in responding pts, there was no expansion of high-risk sub-clones. Conventional karyotyping was performed in 10/37 patients who progressed both pre-treatment and at progression. In 4 pts, CCE was identified at progression, including 17 new abnormalities in one pt. All 4 pts had complex karyotype and del(17p) by FISH pre-treatment and 3 of 4 had evidence of multiple, related, complex sub-clones pre-treatment. Figure 1 shows inferred clonal evolution pattern for one pt.

**Figure 1.**
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.

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

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

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

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

Results: Among the 288 CLL pts treated with lenalidomide, 102 (35%) were previously untreated and 186 had R/R CLL. Ninety two patients (32%) received lenalidomide as a single agent and 196 patients (68%) received in combination with rituximab or ofatumumab. In total, we detected 470 high-confidence somatic mutations in 61 genes in 281 pts (76%). In addition to the well-known arm-level chromosomal abnormalities like del(13q), del(11q), tri(12), and del(17p), we also detected amp(2p), del(6q), del(8p), amp(8q) and tri(19). The most frequently mutated gene was SF3B1 (15%), followed by NOTCH1 (14%) and TP53 (14%) with 13 gene mutations occurring ≥3%. The number of mutations was similar between untreated and R/R pts (median of number of mutations 1 [IQR: 0-2] vs 1 [IQR: 1-2], P=0.44) with increased enrichment of complex cyto geneticities, TP53 mutation and del(17p) in the R/R cohort (p=0.006, p=0.014 and p=0.031, respectively). The pts with unmutated IGHV status had higher number of mutations compared to mutated IGHV (median 1 [IQR: 1-2] vs 1 [IQR: 0-2], P=0.02) with MYD88 mutation and del(13q) being significantly enriched in IGHV mutated pts (p=0.005 and p=0.028, respectively) while NOTCH1 and XPO1 mutations were significantly enriched in IGHV unmutated patients (p=0.035 and p=0.047, respectively).

Pairwise association showed statistically significant co-occurrence between tri(12) and mutations in KAS/BCOR (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(11q), p=0.035), DDX3X (p<0.001), complex karyotype (p=0.035) and del(17p) (p=0.031) were associated with worse OR in R/R group. In the untreated group, del(17p) and TP53 were associated with worse progression-free (PFS) (p=0.002 and 0.003, respectively). In R/R cohort, complex karyotype, del(17p) and mutations in SF3B1 and TP53 were associated with worse OR. In total, we detected 470 high-confidence somatic mutations occurring ≥3%.

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.

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

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

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

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

Results: Overall, 48 samples were analyzed, producing 12,386,554 and 4,506,464 total reads for heavy and light chain, respectively. In addition to filtering out poor quality, incomplete, out-of-frame and unproductive rearrangements and filtered reads, parameters were also filtered by the usage of subset #4-specific V- and J-genes, CDR3 length and landmark residues. Applying these strict criteria resulted in 84.1% (median 401,133 reads/sample) and 90.3% (median 141,549.5 reads/sample) of the total sequences obtained for the heavy and light chain, respectively, passing filters. Clonotype computation was solely based on the filtered reads, and revealed a median of 1332.5 clonotypes/sample (range: 879-3432) for the heavy chains while a median of 202.5 clonotypes/sample (range: 125-395) was evidenced for the light chains. Overall, our longitudinal analysis revealed: (i) a hierarchical pattern of subclonal evolution showing which SHMs were more strongly enriched (positively selected SHMs), (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.

Summary/Conclusions: Detailed molecular immunoprofiling by NGS afforded the possibility to gain novel insights into the pathogenesis of CLL subset #4, thus providing conclusive evidence that these patients continue to acquire SHMs within their IG genes; an observation best explained by a clear role for antigen selection in clonal evolution.
BACKGROUND: During normal hematopoiesis, a coordinated epigenetic and transcriptional programming is necessary to achieve lineage development. B cell differentiation is predominantly related to loss of DNA methylation at the enhancers and promoters of B cell–specific genes; e.g., transcription factors (TFs). In chronic lymphocytic leukemia (CLL), failure of proper epigenetic programming contributes to deregulation of B cell transcriptional programs and results in CLL phenotypes with highly variable outcomes. The mechanisms leading to failed epigenetic programming and to establishment of a CLL epigenome are not well understood. Genomic sites of failed epigenetic 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 CLL and 4 healthy B cell samples (CD19+) were used for DNA methylation profiling using Illumina Epic arrays and for hydroxymethylated DNA immunoprecipitation (hMeDIP) using a monoclonal 5hmC mouse antibody and the NEBNext Ultra DNA Library Prep Kit for analysis on a Illumina HiSeq2000 sequencer. Global 5hmC levels were quantified by dot blots. TET2, and EBFB1 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 IG HV 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 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) and B cell receptor (BCR) signaling, as well as in CD19, CXCR-4, c-Med and IL-3 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 EBFB1 motif at the respective sites in 122 CLL patients. EBFB1 mRNA and protein expression was significantly reduced in the majority of 17 CLL samples compared to HBC. TET2, a potential interaction partner of EBFB1, 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 EBFB1 and TET2 are mechanistically involved in insufficient hydroxymethylation and consequently failed DNA hypomethylation.
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ADDING OBIJNUTUMAB TO IBRUTINIB ENHANCES DEPLETION OF CLL CELLS IN PERIPHERAL BLOOD AND BONE MARROW AFTER 1 & 6 MONTHS COMBINED THERAPY INITIAL RESULTS FROM THE BLOODWISE TAP ICICLLe EXTENSION STUDY


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Background: A major aim of CLL treatment is to eradicate detectable minimal residual disease (MRD). Ibrutinib is an effective treatment for CLL that results in immediate lymphocytosis persisting in most patients for several months. Obinutuzumab is a second-generation anti-CD20 monoclonal antibody which can effect rapid resolution of lymphocytosis and eradication of MRD in some CLL patients. The ICICLLe Extension Study expands on the ICICLLe trial (ISRCTN12693534) 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 previously relapsed/refractory (RR), to receive continuous ibrutinib therapy until confirmed MRD negative remission (<0.01% residual disease) or disease progression. The ICICLLe Extension Study aims to evaluate whether patients achieving MRD-negative remission have better overall survival outcomes compared to patients not achieving this remission.

Methods: The effects on MRD negative status were assessed in 5 groups: Incidence of MRD negative status up to 6 months, number of patients with MRD negative status at 6 months, clinical characteristics of patients achieving MRD negative status at 6 months, clinical characteristics of patients not achieving MRD negative status at 6 months.

Results: Of the 40 patients treated, 31 (78%) achieved MRD negative status at 6 months, 26 (65%) at 12 months, and 24 (60%) of the 40 patients achieved a molecular complete remission by 12 months post-treatment. The time to achieve MRD negative status was shorter in patients achieving complete remission compared to those who did not achieve remission, with a median time to remission of 2.5 months for complete remission and 9.5 months for those who did not achieve remission. The median time to progression was 19 months for patients achieving MRD negative status and 7 months for those who did not achieve remission.

Summary/Conclusions: The results from the ICICLLe Extension Study show that continuous ibrutinib therapy with obinutuzumab is feasible and results in a high incidence of MRD negative status and molecular complete remission. Further study is needed to confirm these findings and investigate the durability of these outcomes.

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

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

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

Methods: 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 IGHV3-21: IGLV3-21 patients had a median TFS of 17 months compared to 44 months in patients with another light chain (P=0.0270). Similarly, IGLV3-21 patients had a shorter median OS (88 months vs +192 months, P=0.0287). We verified these results in 2 independent cohort obtained at diagnosis. In cohort B (n=270), 30 (11%) expressed an IGLV3-21 light chain and 10 (4%) an IGHV3-21 (of which 8/10 also carried the light chain IGLV3-21 rearrangement). Patients with IGLV3-21 had a median TFS/OS of 29/183 months compared to patients without IGLV3-21 who had a median TFS/OS of 88/292 months (P=0.0003/P=0.0142). In cohort C (n=103), 9 (9%) expressed an IGLV3-21 light chain but only 1 (1%) had a heavy chain IGHV3-21. In this cohort, IGLV3-21 patients had a median TFS of 21 months not statistically different from IGHV3-21 patients (28 months) while IGHV3-21 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 IGLV3-21 or IGLV3-21 (with a M or UM IGHV) displayed a prognosis similar to UM patients: median TFS of 12 months (P=0.0270). Patients with another light chain with a IGLV3-21/M (P=0.0001), UM (P<0.0001), IGLV3-21/UM (P<0.0001) and IGLV3-21 (P<0.0001) patients, respectively (Figure 1A). Similar results were observed for OS with a median OS of 292, 88, 174, 90 and 183 months M, IGLV3-21/M (P=0.0005), UM (P<0.0001), IGLV3-21/UM (P<0.0001) and IGLV3-21 (P<0.0001) patients, respectively (Figure 1B). If all IGLV3-21 (n=48) were considered independently of their heavy chain, IGLV3-21 median TFS (24 months) was similar to UM patients (36 months, P=0.5824) and statistically different from M patients (129 months – P<0.0001, Figure 1C). Similar results were observed for OS (Figure 1D).

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

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

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

Methods: Minimal residual disease (MRD) was assessed in bone marrow using ≥4-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-neg-ative 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-initiated venetoclax, 2 and 6 months after ALC >5x10⁹/L, and achieved partial remissions. The 10 patients with MRD-negativity in the bone marrow who remain in follow-up have a median duration of ongoing response off venetoclax of 13 months (range: 3–34).

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

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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. We investigated NOTCH1mut as a predictive factor in the context of chemoimmunotherapy.

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 1–14-16, 18), NOTCH1mut vs FC+ofatumumab (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 (all coding exons), SF3B1 (exon 14–16, 18), NOTCH1 (exon 34) and XPO1 (exon 15, 16). All mutations with a variant allelic fraction >5% were considered significant.

Figure 1.

Results: In total we identified 365 mutations across the 9 genes in 202 of 325 patients (62.2%), with incidences of SF3B1mut 19.7%, TP53mut 18.8%, NOTCH1mut 16.3%, ATMmut 13.8%, XPO1mut 11.4%, BIRC3mut 4%, EGR2mut 3.1%, FBXW7mut 2.7% and MYD88mut 0.9%. We identified a variety of associations of mutational subgroups with genetic, clinical and laboratory parameters, such as TP53mut with del17p (p<0.01), NOTCH1mut, FBXW7mut and BIRC3mut with +12q (p<0.01, p=0.01 and p=0.05) and ATMmut with del11q (p<0.01). XPO1mut and ATMmut associated with unmuated IGHV, CD79B expression on cell surface measured via flow cytometry was lower in ATMmut patients, whereas CD220 expression did not differ among the different mutational subgroups. TP53mut, EGR2mut and SF3B1mut patients had worse overall response to therapy (88% p<0.01, 50% p=0.02 and 72% p=0.05 respectively, vs 81% overall). Similar to the full analysis set, FC0 as compared to FC resulted in significant improved PFS (median 28.1 vs 18.8 months, HR=0.67, p<0.01), TP53mut and XPO1mut were adverse prognostic factors for PFS (HR 1.93, p<0.01 and HR 1.85, p<0.01 respectively), but only TP53mut for decreased OS (HR 2.11 p<0.01). All other mutations, in particular SF3B1mut and NOTCH1mut, did not significantly impact PFS or OS. To identify factors of independent clinical
impact, we performed multivariable Cox regressions for PFS and OS including treatment, IGHV status and all cytogenetic and mutational subgroups. For PFS, the following independent prognostic factors were identified: FCO therapy (HR 0.64 p<0.01), del17p (HR 5.08 p<0.01), unmutated IGHV (HR 2.0 p<0.01), TP53mut (HR 1.75 p<0.01) and XPO1mut(1.86 p<0.01). Del17p (HR 4.79 p<0.01), unmutated IGHV (HR 1.69 p<0.04) and TP53mut (HR 1.76 p<0.03) were identified as independent prognostic factors for OS. With focus on the predictive value of gene mutations, we found a beneficial effect of the addition of ofatumumab to chemotherapy irrespective of TP53 mutation (HR 0.52 p=0.02 for TP53mut and HR 0.68 p=0.02 for TP53mt). Regarding NOTCH1, ofatumumab 4G11 was only beneficial in NOTCH1mt but not in NOTCH1mut patients (HR 0.64, p<0.01 and HR 0.86, p=0.67) (Figure 1).

Summary/Conclusions: In the COMPLEMENT-2 trial evaluating FCO against GC in relapsed/refractory CLL patients, we found TP53mut and XPO1mut but not SF3B1mut or NOTCH1mut as independent prognostic factors for PFS. Notably, the addition of ofatumumab as 4G11 to FCO treatment was beneficial among NOTCH1mt but not among NOTCH1mut patients indicating NOTCH1 mutation status as a predictive marker in the context of type 1-2CD20 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 II anti CD20 monoclonal antibody. A randomized Phase III trial in 1L CLL pts showed that G significantly improved progression-free survival (PFS) and CR rate compared with R, when used in combination with chlorambucil (Goede 2014). B plus G (BG) was evaluated in a subgroup of CLL pts in the GREEN study (Stilgenbauer 2015).

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

Methods: 102 pts with previously untreated CLL received BG, consisting of 6 cycles of G (cycle [C] 1: 100mg day (D1), 900mg D2, 1000mg D3 and D15; C2– 6: 1000mg D1 and B (80mg/m2; C1, D2 and C3; C2–6, D1 and D2). Each cycle was 28 days. The primary endpoint was CR assessed using iwCLL criteria. Secondary endpoints included ORR, PFS, overall survival, and minimal residual disease (MRD). Median follow-up at the time of analysis was 11.0 months. Results: Median pt age was 61 yrs (range 35–90); 68.6% were male; 44.1% had Rai stage 3–4. For evaluable pts, IgVH status was 32.9% mutated and 67.1% unmutated. Incidences of trisomies 12, normal cytogenetics, and deletions of 13q, 11q, and 17p were 23.4%, 37.5%, 17.2%, 15.6%, and 6.3%, respectively. Investigator-assessed CR rate was 49.0% (95% CI 39.0–59.1) and ORR was 89.2% (95% CI 81.5–94.5) after 6 cycles. MRD negativity in blood, as measured by next-generation flow cytometry, was achieved in 42.7% of pts at the end of induction response assessment and in 75.5% of pts at any time following treatment. MRD negativity in bone marrow (BM) was 60.8% in pts with BM samples. The most common adverse events (all grades [Gr]) were infusion reactions (72.5%), nausea (52.0%), pyrexia (36.3%), neutropenia (34.3%), fatigue (34.3%), constipation (26.5%), and rash (26.5%). The most common Gr 3–4 adverse event was neutropenia (26.5%). Incidence of Gr 3–4 infections was 11.8%. Incidence of tumor lysis syndrome was 4.9% (all Gr 3). Three pts died; none were deemed related to study treatment or CLL by investigators.

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

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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 nationwide 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=.005). Ten-year RS (95% confidence intervals) was impressive for pts age 19-59, namely 92% (88% - 96%) and 98% (94% - 100%; P=.176) in the first and last period, respectively (Figure 1a). Most of the significant improvement was observed in pts age ≥60. More specifically, 10-year RS for pts age 60-69 increased from 82% (71% - 92%) to 99% (89% - 100%; P=.009; Figure 1b), and for pts age ≥70 from 67% (49% - 86%) to 80% (72% - 88%; P=.03; Figure 1c) between the first and last periods. In addition, older age (P<.001), but not sex (P=.058), was associated with higher excess mortality.

Figure 1.

Summary/Conclusions: The incidence of HCL remained stable during a 26-year period in the Netherlands. RS for pts diagnosed in the period 2001-2014 eventually reached a plateau, indicating that by then their survival is comparable to that of the general population. Survival was already excellent for younger patients throughout the entire study period. Survival improvement was most pronounced for pts 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.

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CUMULATIVE ILLNESS RATING SCALE PROVIDES PROGNOSTIC INFORMATION BEYOND THE INTERNATIONAL PROGNOSTIC INDEX FOR CHRONIC LYMPHOCYTIC LEUKEMIA: AN ACROSS-TRIAL ANALYSIS BY THE GCLLSG

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

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

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

Results: Median age was 64 years; 69% of patients were males, and 50% had EGOG performance score of 1 or higher. Complete information on age, Binet stage, beta-2-microglobulin, 17p deletion / TP53 mutation, IgHV mutational status was available in 1761 of the 2158 patients. Distribution of CLL-IPI risk groups was as follows: 275 (16%) low risk, 653 (37%) intermediate risk, 712 (40%) high risk, 121 (7%) very high risk. The median total CIRS score was 3 (range 0-22); 81% of the patients had a total CIRS score of at least 1 and 28% of greater than 6. Comorbidities were most frequently captured under the following categories: cardiovascular, lung, gastrointestinal, psychiatric, and metabolic comorbidities. The frequency of each comorbidity was as follows: coronary artery disease (23%), chronic obstructive pulmonary disease (22%), diabetes (21%), hypertension (19%), and HIV infection (13%).

Figure 1.

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

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

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Background: GALTON was an open-label, parallel-arm, non-randomized, multicenter, Phase 1b study (NCT01300247) investigating safety and preliminary efficacy of G-F/C 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-F/C 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 (fluorarabine 250mg/m2 IV and cyclophosphamide 250mg/m2 IV D2–4 C1, D1–3 C2–6) or B (90mg/m2 IV D2–3 C1, D1–2 C2–6). Each cycle was 28 days. The primary endpoint was safety and tolerability of G-chemotherapy.

Results: 21 pts were enrolled in the G-F/C 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-F/C) and 17 mo (G-B). 37% of pts were alive in follow-up: G-F/C (n=18; 2 lost to follow-up) and G-B (n=19). 7 events of progressive disease occurred in each arm, and 1 pt per arm died due to an adverse event (AE; G-B: respiratory failure; G-F/C: 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-F/C, 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-F/C 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 leukenpwa/leuk/leup, small cell lung cancer and Gr4 pneumothorax, 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-F/C 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 neutrophils (G-F/C: 2/19 pts [10.5%]; G-B: 0/20 pts). At 36 mo follow-up, 9/19 pts (47.3%) in the G-F/C arm had recovered, 3/19 (15.8%) were still depleted, and 7/19 did not have data available. In the G-B arm, 6/20 pts (30%) had recovered, 1 was still depleted, and 13/20 had no available data. In a single center exploratory analysis, 9 pts (G-F/C) underwent 4-color flow cytometry for assessment of peripheral blood for minimal residual disease (MRD) 8–14 mo after therapy; all were negative. 8 of these pts (G-F/C) were MRD-negative by 4-color flow cytometry were also tested with the ClonoSEQ immunoglobulin sequencing assay: 4 were MRD-positive and 4 MRD-negative. 4 pts were MRD-positive by both assays remain in remission, while 2/4 pts who were BCR-positive by ClonoSEQ died after follow-up, one of Richter’s transformation complicating with pneumonia and the other related to MDS. Another pt who was MRD positive by ClonoSEQ underwent allogeneic stem cell transplantation and remains in remission.

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

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THE PROGNOSTIC SIGNIFICANCE OF CLL-IPi AFTER REDUCED INTENSITY CONDITIONING ALLOGENIC 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 outcome following RIC SCT, including the CLL-IPi.

Methods: This is a retrospective analysis of all CLL patients who underwent RIC-SCT at Mayo Clinic between 2006-2013. The study was approved by the Institutional Review Board. The prognostic value of several CLL, patient and transplant related variables were analyzed. Continuous variables were reported as median (IQR) and categorical variables as absolute number and percentage. Survival was estimated using the Kaplan Meier and Log Rank tests.

Results: Between 2006 and 2013, 50 patients with a median age of 56 years old underwent RIC-SCT for the treatment of CLL. The median time from diagnosis to RIC-SCT was 4.7 (0.6–22.9) years. Fourteen (28%) patients had 17p deletion at time of transplantation. CLL-IPi prognostic score calculated prior to transplant was intermediate in 30%, high in 42% and very high in 28% of patients. Disease status at the time of transplant was partial or complete remission in the majority of patients (39 patients, 78%). The overall transplant related mortality (TRM) was 6% and the 5-year non-relapse mortality was 14%. Relapse rates at 5 years were 54%. Acute graft versus host disease (GVHD) developed in 30 (60%) of patients and chronic GVHD was noted in 32 patients (64%). We evaluated the impact of CLL characteristics, disease status, and patient and transplant characteristics on clinical outcomes. Development of chronic GVHD post-transplant was the dominant predictor of both disease-free survival (DFS) (HR 0.29, 95% CI=0.10-0.69, P=0.006) and OS (HR 0.40, 95% CI=0.01-0.57, P=0.001, Figure 1A). Very high CLL-IPi risk category (28% of patients) was associated with high relapse rates (82%) post RIC-SCT. DFS was also different between different CLL-IPi categories (18.2% in very high 52.9% in high vs 66.7% in intermediate, p=0.04, Figure 1B). However, there was no significant difference in overall survival suggesting potential benefits from novel therapies in relapsed patients. Given that development of chronic GVHD was the most significant predictor for OS, we evaluated what pre-treatment, patient and disease (including CLL-IPi), and transplant characteristics were predicted for subsequent development of chronic GVHD. ZAP70 over expression (OR 0.09 [95% CI 0.01-0.79], p=0.03), disease status at transplant (progression versus remission OR 0.22 [95% CI 0.05-0.92], p=0.038), and alemtuzumab exposure within 3 months of transplantation were associated with lower rates of chronic GVHD (OR 0.08 [95% CI 0.01-0.79], p=0.03). CLL-IPi was not a significant predictor for the development of chronic GVHD in our analysis.

Figure 1.

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

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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. & Pfirrmann M. et al., ASH 2016). In addition, biomarkers like NK-cells and CD86+ cells (Ilander M. et al. & Schütz C., Leukemia 2017) seem to be of impact. ABCG2, OCT1 and ABCB1 are known to play a crucial role in acquired pharmacokinetic drug resistance and DMR in the context of nilotinib, imatinib and dasatinib. The influence of these mechanisms have not yet been analyzed for their correlation with TFR.

Methods: Expression measurements were performed by qRT-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) immunocassay was used for the quantitative measurement of HLA-G1 and HLA-G5 soluble forms in EDTA-plasma samples.

Results: The frequency of the G*01:03 allele was significantly associated to OCT1 and the efflux transporters ABCG2 and ABCB1 (MDR1) that have been quantified in order to investigate their impact on TFR. As all patients are in DMR, we investigate whether these transporters confer a constitutional disfunction discontinuation (HR=2.1, 95% CI: 1.06-4.05).

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. Aims: We investigated the potential role of HLA-G polymorphisms and soluble HLA-G molecules in susceptibility to chronic myeloid leukemia (CML), as well as in achievement and maintenance of deep molecular remission (MR4.5) in 68 patients treated with tyrosine kinase inhibitors (TKIs).

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

Results: The frequency of the G*01:03 allele was significantly associated to HLA-G1 and HLA-G5 soluble forms in EDTA-plasma samples. The predictive significance of the efflux and influx channel transcript levels was quantified 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 (87% inmatinib 1st line treatment), showing a relapse rate of 46%. Median MR4 and TKI treatment duration was 4.3 and 7.8 years respectively. The majority of patients were positive for the e14a2 transcript (e14a2+26: 83%, e13a2+ e14a2+ 9%). The mean expression of OCT1 and ABCB1 between ‘relapse’ and ‘no-relapse’ patients showed no significant difference (p=0.99 and p=0.66), whereas 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=0.004). 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: 1.06-4.05).

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.

Figure 1.

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

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

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Background: ENESTop (NCT01698895) is evaluating the ability to stop treatment and remain in TFR in pts with CML-CP who achieved a sustained deep molecular response (MR) after switching from imatinib (IM) to NIL. In the primary analysis, 57.9% of pts (73/126) who stopped treatment remained in TFR (no loss of major MR, BCR-ABL1 ≤0.1% on the International Scale (IS)), with a confirmed loss of MR4 [BCR-ABL1 ≤0.01%], and no treatment reinitiation at 48 wk.

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

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

Results: At 96 wk phase, 67 of the 126 pts (53.2% [95% CI, 44.1% - 62.1%]) who entered the TFR phase remained in TFR. Four pts who were in TFR at 48 wk reinitiated NIL due to confirmed loss of MR4 at 60, 72, 90, and 96 wk, respectively. Two other pts discontinued from the study between 48 and 96 wk due to pregnancy (last BCR-ABL1 of 0.0035% at 60 wk) and pt decision (maintained MR4.5 through 90 wk), respectively. Based on Kaplan-Meier analysis, the median duration of treatment-free survival has not been reached and the curve appeared to plateau (Figure 1). Of 56 pts who reinitiated NIL by the data cutoff, 52 (92.9%) regained MR4 and MR4.5, and the time by which 50% of pts regained MR4.5 was 12.0 and 13.1 wk, respectively. The time by which 50% of pts regained MR4 was shorter for pts on first-line vs second-line therapy which may be due to a confirmed loss of MR4 (n=22; 11.0 wk) vs loss of MMR (n=34; 16.0 wk). Two of the 4 re-treated pts who did not regain MR4 were ongoing in the treatment reinitiation phase (duration, 8.9 and 6.9 wk, respectively), the other 2 discontinued from the study before 48 wk, 1 due to not regaining MMR (retreatment duration, 20 wk) and 1 due to an adverse event (AE) after regaining MMR. Two deaths occurred after the first 48 wk of TFR, both in post-treatment follow-up: 1 due to cardiopulmonary failure 111 days after pt discontinued retreatment due to an AE, and 1 due to adenocarcinoma 77 days after pt discontinued retreatment due to initiation of chemotherapy for secondary malignancy. Among pts who remained in TFR (n=73), rates of all-grade AEs, grade ≥3 AEs, and grade 4 AEs were 82.2%, 63.0% and 63.0% during the first and second 48 wk of TFR, respectively, vs 79.5% during the consolidation phase. Rates of musculoskeletal pain-related AEs were 47.9% and 15.1% during the first and second 48 wk of TFR, respectively, vs 13.7% during the consolidation phase.

Summary: Among 96-wk analyses from ENEStop showed stability of the TFR rate, with few pts reinitiating treatment between 48 and 96 wk after stopping second-line NIL. Rates of overall and musculoskeletal pain-related AEs decreased in the second 48 wk of TFR vs the first 48 wk. Overall, these results demonstrate the durability of TFR after stopping NIL in pts who achieved a sustained deep MR after switching from IM to NIL.

Figure 1.

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

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

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

Methods: Differentiating 3T3-F442A murine adipocytes were incubated with clinically relevant concentrations of NILO (1-10µM) and IMA (5µM); in the presence or absence of telmisartan (1-10µM), an angiotensin receptor blocker with potential beneficial effects on insulin sensitivity and lipid homeostasis. Cytoxicity and adipogenesis were assessed by MTT assay and Oil Red O staining, respectively. Expression of adipogenic genes were assessed using qPCR and targeted receptor gamma (PPARγ), lipin1 (LPIN1), sterol regulatory element-binding protein 1 (SREBP1) and glucose transporter 4 (GLUT4) were investigated by quantitative PCR and secreted adiponectin was measured by ELISA. Plasma samples were collected from 30 CML patients on either NILO (first line, n=6; n=8 for first, first-line, n=15) at baseline and at 3 and 12 months of therapy, and adiponectin was measured by ELISA. Data are presented as mean ± SD for 20µM incubations but full concentration response relationships were measured.

Results: Neither NILO nor IMA were cytotoxic to the adipocytes at clinically relevant concentrations. A dose dependent reduction in lipid accumulation was observed for NILO (for 20µM, 0.76 ± 0.005 absorbance units; p<0.01) but not IMA (0.98 ± 0.007), compared to vehicle control. NILO, but not IMA, decreased in the second 48 wk of TFR vs the first 48 wk. Overall, these
Summary/Conclusions: A BCR-ABL1 transcript level measured at 1 month after initiation of a TKI may be used as an early indicator to reliably predict the MMR achievement by 12 months in patients with CP-CML. The level obtained at 3 months appears to accurately predict the MMR. Further studies are needed to evaluate the association between the transcript level at 1 month and long-term clinical outcomes.

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

Background: A BCR-ABL1 transcript level at 3 months after the initiation of imatinib has shown to predict the long-term clinical outcome 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 (MMR) achievement by 12 months, and to compare the patterns of molecular response (MR) to a TKI therapy between good and poor responders using a nonlinear model.

Methods: The clinical data were collected from the 178 patients with newly diagnosed CP-CML who were treated with a TKI at Seoul St. Mary’s Hospital. BCR-ABL transcripts were obtained at baseline, and 1, 3, and 6 months after the initiation of a TKI. The levels were reported as the percent ratio relative to the control gene ABL1 in accordance with the International Scale (BCR-ABL1/ABL1×100%). A confirmed MMR was defined as a BCR-ABL1/ABL1≤0.1% on two consecutive occasions. The predictability of the levels at baseline, and 1 and 3 months post TKI therapy for the achievement of a confirmed MMR by 12 months was evaluated using a logistic regression method with a receiver operating characteristic (ROC) analysis. The areas under the ROC curve (AUCs) were calculated to quantify the predictability. In addition, the patterns of molecular responses over time were described by a nonlinear model by the model-derived parameters between the patients who achieved a confirmed MMR by 12 months (“good responders”) and who did not achieve the MMR (“poor responders”).

Results: Of 178 patients, 67 achieved a confirmed MMR by 12 months but 111 did not. At baseline, the transcript level was not useful to predict the achievement of a confirmed MMR by 12 months. At 1 month post therapy, the levels measured at 1 month significantly (p < 0.0001) predicted the MMR with an AUC of 0.77. The patients with the level of 38% or less at 1 month had a better chance to achieve the MMR. By 3 months post therapy, the transcript level measured at 3 months (p < 0.0001) accurately predicted the MMR with the AUC of 0.94. The patients with the level of 0.48% or less at 3 months had a better chance to achieve the MMR. A nonlinear sigmoid model was used to fit the transcript data from 149 patients as follows: MR=MR0 [1 – tγ/(t50γ+tγ)]; where MR0 is the predicted molecular response at baseline; t, time post TKI initiation; y, slope factor; t50, time required to achieve 50% reduction in MR. Statistically significant differences were observed between the good and poor responders in the median values for the model-derived parameters of MR0 (73.3% vs 82.2%; p=0.003), y (4.98 vs 3.32; p < 0.0001) and t50 (0.952 month vs 1.12 month; p=0.7).

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

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Abstract withdrawn.

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

Background: Digital PCR (dPCR) generates an absolute read out that is largely robust to variations in PCR efficiency and should reduce the requirement for standardisation by laboratory-specific conversion factors. DPCR is an appealing technology for quantitative detection of specific mutations with simultaneous measurement of the reference gene and is particularly suitable for minimal residual disease (MRD) diagnostics in chronic myeloid leukaemia (CML). However, a limitation of the dPCR assays compared to standard quantitative PCR (qPCR) is the background (termed lower limit of blank, LoB) of 1 or 2 positive droplets for BCR-ABL (Franke et al., ASH 2015, Cross et al., Leukemia 2016). The resulting false positive rate (FPR) limits the sensitivity and the ability to detect deep molecular remissions. This has hindered the definition of the lower limit of detection (LoD). We report here on the LoB and the LoD of a newly developed assay.

Aims: Determination of LoB, LoD, linearity and precision of an optimized BCR-ABL digital PCR assay.

Methods: The assay was developed by Bio-Rad laboratories and consisted of a reverse transcriptase reaction followed by a duplex PCR detecting ABL and both the b2/a2 and b3/a2 transcripts of BCR-ABL. Digital droplet PCR was performed using the Bio-Rad QX200 system.

Results: The specificity was >95% for both BCR-ABL and ABL in NTC and wild type samples. Extensive NTC analysis yielded no false positives for BCR-ABL PCR (n=0/176; LoB=0) and 1% false positives in ABL PCR (n=2/176; 1-2 positive droplets, LoD=0). The BCR-ABL assays of healthy donors were positive in 2% (n=5/234) with 1 positive droplet/positive sample. For 2 wells analysis, the detection rate for BCR-ABL for MR5 and MR6 was 100 and 88%, resulting in a LoD between 6 and 3 BCR-ABL copies/2 wells. Although the ABL copy number was only 20000 for 2 wells, the MR5-6 detection rate was 42%. However, in a merged analysis, the detection rate was 100% for MR5 and 67% for MR6 with an average of 6 and 2 BCR-ABL copies/4 wells (see Table 1). The MR5-6 sample was analyzed repeatedly, with 59 of 60 replicates evaluable. A total of 89 BCR-ABL copies (1.51/replicate) and 4329,846 ABL copies (73387/replicate) were detected, resulting in a ratio of 0.0021 (MR4-6). The false negative rate was below 3% when combining any pair of replicates, indicating an LoD of 4-5 for assays carried out in duplicate.

Table 1

<table>
<thead>
<tr>
<th>Disease</th>
<th>LoB</th>
<th>LoD</th>
<th>Linearity</th>
<th>Precision</th>
<th>Detection rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL</td>
<td></td>
<td></td>
<td>n=176</td>
<td>1%</td>
<td>100% (n=0)</td>
</tr>
<tr>
<td>ABL</td>
<td></td>
<td></td>
<td>n=176</td>
<td>1%</td>
<td>100% (n=0)</td>
</tr>
<tr>
<td>MR5</td>
<td>0.0021</td>
<td>0.0021</td>
<td>0.0021</td>
<td>0.0021</td>
<td>100% (n=0)</td>
</tr>
<tr>
<td>MR6</td>
<td>0.0021</td>
<td>0.0021</td>
<td>0.0021</td>
<td>0.0021</td>
<td>88% (n=2)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: We report here on an optimized digital PCR assay with a LoB of zero and a LoD of 3 copies/2 wells. This highly sensitive and specific assay allows accurate detection of MRD in BCR-ABL positive diseases with a detection rate of 100% for MR5 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 risk of cytogenetic Complete Response (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 of dying 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=0.02) and the low risk group (1%, p=0.001). Between the intermediate and low risk group no statistically significant difference in risk of dying from CML was observed. A subgroup analysis of only imatinib treated patients showed similar results.

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

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FINAL STUDY RESULTS OF DISCONTINUATION OF DASATINIB IN PATIENTS WITH CML WHO MAINTAINED DEEP MOLECULAR RESPONSE FOR LONGER THAN ONE YEAR (DADI TRIAL) AFTER THREE YEARS OF FOLLOW-UP


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Background: A second-generation tyrosine kinase inhibitor (TKI), dasatinib, is more potent in inhibiting BCR-ABL than imatinib. We had previously reported an interim analysis of 63 patients with CML-CP who had discontinued dasatinib treatment after maintaining a deep molecular response (DMR) for more than a year (Lancet Haematology, 2015; 2 (12):e528-35) and demonstrated that dasatinib could be safely discontinued in patients with a DMR for at least 12 months. Longer follow-up results would clinically be more critical in the treatment of CML.

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

Methods: The eligibility criteria for pre-registration included CML-CP patients, 15 years or older, receiving dasatinib treatment as the second-line or subsequent therapy after imatinib. All participants gave written informed consent.

In this trial, DMR was defined as “no detectable BCR-ABL1 transcript determined using the international scale-based RQ-PCR at a single central laboratory (BML Inc., Tokyo; the cutoff corresponded to BCR-ABL1 0.0069% IS or molecular response (MR) 4.0).” Patients who showed a sustained DMR for 1 year (1-year consolidation phase) were subsequently included in the dasatinib-discontinuation stage. RQ-PCR was performed monthly for the first 12 months, and then every 3 months for the second year, and every 6 months for the third year, after discontinuing dasatinib. Relapse was defined as any positivity of BCR-ABL1 transcript by RQ-PCR even at one analysis point. In the present study, we assessed the estimated overall treatment-free remission (TFR) after discontinuing dasatinib, with a 1-year follow-up period.

In addition, we also evaluated the impact of immunological profiles, including the cell counts of T and NK cell subsets in the peripheral blood throughout the 1-year consolidation phase, on TFR.

Results: Sixty-three patients were included in the dasatinib-discontinuation stage. The total follow-up duration was set as 36 months after dasatinib discontinuation. After molecular relapse, 1 patient showed fluctuating BCR-ABL1 transcript values below MR 4.0 without restarting dasatinib therapy. A total of 35 out of 63 patients showed molecular relapse. No new cases of second diagnostic translocation were observed. The estimated overall TFR rate was 44.4% (95% confidence interval [CI], 32.0-56.2) at 36 months. A high count of NK-cell phenotypes (CD3-CD56+ cells ≥539 cells/μl and CD16+CD56+ cells ≥506 cells/μl) and a low count of gd+ T-cells (<120 cells/μl) were detected to be significant factors affecting molecular relapse in the interim analysis; these showed sustained significance as predictors of a favorable TFR (P=0.0475, 0.0202, and 0.0093, respectively).

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

P264

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

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

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

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

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

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

P265

BUILDING HUMAN BONE MARROW-LIKE MODELS TO STUDY NICHE INTERACTIONS

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

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

Methods: Towards successful implementation of a human vascular system in vitro scaffold is mandatory. We (i) tested different scaffold compositions (biodegradable cell to protein scaffolds (BCP) vs tricalcium phosphate (TCP)); (ii) scaffold shape (particles vs tubes); (iii) different types of matrigel for cord blood-derived endothelial progenitor cells (CB-EPCs) embedding.

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

Summary/Conclusions: Thus, with the addition of human CB-EPCs and BM stromal cells, our scaffold systems now simulate both endothelial and vascular niches of the BM, thereby more closely recapitulating the human hematopoietic microenvironment.

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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 in situ, playing fundamental regulatory roles in hematopoietic development. Recent evidence suggests that tissue regions around BM venous microvessels (termed sinusoids), which are enriched for mesenchymal CXCL12-abundant reticular cells (CARc), serve as the principal regulatory niches for HSCs as well as other hematopoietic progenitor populations. Despite this proposed role as a source of 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 tech-niques to perform a detailed 3D quantitative and structural analysis of the BM stromal infrastructure, with a special focus on sinusoidal microvasculature and the CAR cell mesenchymal component, both of which are essential regulators of HSC maintenance.
**P267**

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

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

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

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

**Results:** Six unique VDJ sequences, all from the same donor, carried a templated insertion in-frame (E=10−3 – 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 sequences positioned closely together on chromosome 22. Somatic hypermutation correlated strongly between the the IGHV segment and the templated insertions (r=0.9944; p<0.001). All templated insertions harboured cryptic RSS sites at their termini. All three IgG VDJ carrying templated insertions and the IgG rearrangement with the 22q13 deletion template insertion gave rise to detectable surface immunoglobulin following coexpression with at least one light chain in the panel. The IgG VDJ carrying the LAIR1 templated insertion produced no detectable surface immunoglobulin. In contrast, the VDJ sequences carrying LAIR1 templated insertions as described by Tan et al. could be expressed with the majority of the light chains. The IgA rearrangement remains to be tested in this system.

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

**P268**

**TARGETING THE CASPASE / NOX2 AXIS TO MODULATE MACROPHERAGE 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, caspase-10 and caspase-2 knockout mice, which were treated with bleomycin to induce pulmonary fibrosis.

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

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

Figure 1.

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

Results: Gene expression analysis and single cell RQ-PCR. CRISPR/Cas9 technology. The loss of CXCL13 had no effect on MM in vitro growth or drug sensitivity. However, mice inoculated with CXCL13-silenced MM cells developed significantly weaker BM disease compared to mice receiving the non-manipulated cells. Reduced tumor load correlated with decreased numbers of M2c-MΦ in BM, decreased bone disease, and lower expression of OC-associated genes. Finally, the presence of CXCL13 in primary MM samples was validated. 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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Figure 1.
development. We thereby report for the first time that the clonal origin of blood is much more complex than previously thought, with hundreds of precursors contributing to the establishment of the mammalian blood system at multiple stages of ontogeny.

Methods: We employed generated

Results: We verified efficient ablation of Cebpg on mRNA and protein level in bone marrow and spleen of Cebpg KO mice. Analysis of peritoneal cavity of WT and Cebpg KO mice showed similar frequency and numbers of mast cells in steady state conditions. However, Cebpg deficient mice exhibit increased number of peritoneal mast cells after LPS stimulation in comparison to WT control littermates. Surprisingly, mice lacking Cebpg presented defective peritoneal mast cell repopulation. Since mast cells are scarce and difficult to isolate from in vivo models, we employed BM-MCs to investigate the effects of Cebpg ablation in mast cell development and function. We observed that bone marrow from Cebpg KO mice generated reduced number of BM-MCs in comparison to WT controls. Functionally, we demonstrated that deletion of Cebpg reduced mast cell migration towards antigen, SCF or PGE, and impaired degradation upon FcεRI-mediated activation. Further, BM-MCs exhibit increased expression of C/EBPα in the absence of C/EBPγ.

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

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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: VCA1+Flt3 (MPP1); VCA1-Flt3+ (MPP2) and VCA1-Flt3+Lr7 (CLP), were tagged with different barcodes carried by a collection of lentivirus and transplanted in mice. Barcoded cells in recipient mice were analyzed by flow cytometry. Transcriptome-wide profiling of 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 was used to identify, by clustering 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 assessed by searching for co-expressed transcription factors and enriched binding sites in their promoters. Their groupings 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**

**P275**

**LONG-TERM OUTCOME OF PATIENTS WITH NODULAR LYMPHOCYTE-PREDOMINANT HODGKIN LYMPHOMA TREATED WITHIN THE RANDOMIZED HD7-HD15 TRIALS: AN ANALYSIS FROM THE GERMAN HODGKIN STUDY GROUP**

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**Background:** Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare entity accounting for approximately 5% of all Hodgkin lymphoma (HL) cases. Pathological and clinical features differ from classical HL (cHL). Pathologically, the malignant lymphocyte predominant (LP) cells stain consistently positive for CD20 and are negative for CD30. Clinically, NLPHL often has a rather indolent course. Despite 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: HD7, 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 AVD 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 relapsed disease or relapsed during the course of follow-up (primary disease progression: 15 patients, early relapse: 6 patients; late relapse: 66 patients). Second malignancies including histological transformation into aggressive B-cell non-Hodgkin lymphoma (NHL) occurred in 48/471 patients (10%) (solid tumor: 25 patients; leukemia: 7 patients; NHL: 13 patients; unspecified malignancy: 4 patients). For all 471 patients included in the present analysis, the 8-year overall survival (OS) rate was 92.2% (95% CI: 89.1–94.5). On average, patients were 56 years old at diagnosis.

**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 the patients were not cured and due to second malignancies or other treatment-related late effects. Thus, future clinical trials including NLPHL patients should evaluate whether it is possible to reduce the treatment intensity without compromising efficacy. This goal may be achieved by the partial replacement of conventional chemotherapy by targeted drugs such as anti-CD20 antibodies as well as the reduction of RT fields and doses.

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**P276**

**ADVANCED HODGKIN LYMPHOMA IN THE EAST OF ENGLAND CANCER NETWORK: A 10-YEAR COMPARATIVE ANALYSIS OF OUTCOMES FOR ABVD AND ESCALATED-BEACOPP TREATED PATIENTS 16–59 J. Russell1,*, A. Collins1, A. Fowler1, M. Karanth2, C. Saha1, V. Shyamsunder1, S. Docherty1, K. Maw1, J. Padayatty1, A. Hodgson1, J. Wimpenny1, S. Sadullah1, N. Grigoropoulos1, B. Utenthal1, 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 HD10 trial results in 2009, escalated-BEACOPP (escB) was introduced by some UK centres to improve disease control in poor-risk patients.

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

**Methods:** We collected data retrospectively from 8 hospitals in the East of England Cancer Network from a referral population of 2.64 million (incidence: 0.95 cases per 100,000). Six of the 8 centres introduced escB for poor-risk patients, as determined by physician and patient choice; 44 patients were treated with escB, 202 with ABVD, 3 with alternative regimens, and 1 died pre-treatment. The median age at diagnosis was 35 years (IQR: 22–48) and the median follow-up was 9.5 years (IQR: 1.10–24.89). A total of 43 deaths were recorded for all patients with a minimum follow-up of 18 months from diagnosis were included. The 5-year PFS and OS were estimated using the Kaplan-Meier method, and subgroups were compared using the standard log-rank test.

**Results:** We identified 250 patients (stage IA bulk≥Vb) treated in the East of England Cancer Network from a referral population of 2.64 million (incidence: 0.95 cases per 100,000). Six of the 8 centres introduced escB for poor-risk patients, as determined by physician and patient choice; 44 patients were treated with escB, 202 with ABVD, 3 with alternative regimens, and 1 died pre-treatment. The median age at diagnosis was 35 years (IQR: 22–48) and the median follow-up was 9.5 years (IQR: 1.10–24.89). A total of 43 deaths were recorded for all patients with a minimum follow-up of 18 months from diagnosis were included. The 5-year PFS for all patients was 82% and 5-year OS was 92%. There was evidence of a physician–patient preference to treat poor-risk patients with escB, as a greater proportion of escB patients had a high international prognostic score (IPS 3+) than in ABVD patients (escB 75% vs ABVD 38%, p<0.0001). For the whole cohort, PFS was better for patients treated with escB compared with ABVD (5-year PFS 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 84%; HR 6.9 (95% CI: 3.14–24.89), p<0.001, 5-year OS 92% vs 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 infertility is an important consideration for escB patients. In our 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.
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 treatment were higher in the imaging group (27, 67.5%) compared with the historical group (19, 44.2%; p=0.032). The 3-years DFS was 75% in the imaging group and 36% in the historical group, p=0.02.

Summary/Conclusions: This is the first prospective case-control study using SMAP-US plus CXR to monitor patients with advanced stage HL. We show that SMAP-US plus CXR is a valuable tool to improve follow-up in patients with a high risk of recurrence. Our data indicate that the early detection of HL recurrence allows to begin rescue therapy in patients with a more limited disease and, consequently, increase its effectiveness in terms of probability to response and DFS.

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

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Background: Whilst cHL is seeing increasing ‘cure’ rates, a cohort of patients remain who, due to multiple relapses, require 3rd or 4th line (4L) lines of drug treatment. Real world treatment patterns for RRHL patients are currently less understood.

Aims: To understand the drug treatment patterns of cHL patients in 3rd or later line treatment.

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 as positive. The most commonly prescribed 3rd line drug treatment was a brentuximab-vedotin (BV) based regimen (35%). BV use was significantly different across the markets; Canada (34%), France (35%), Germany (30%) and the UK (44%) (p=0.010). The next most commonly prescribed 3rd line treatment 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, 38% confirmed as positive. 30% of 4th line patients received a BV based regimen – BV use across markets was significantly different; Canada (20%), France (38%), Germany (23%) and the UK (36%) (p=0.007). At 3rd line this cohort had 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 regimen at 3rd line. There also appears to be no clear standard of care at 3rd line, again highlighted by use of a range of regimens and PD-1’s.

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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. Case 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 (78.8%), the most common was nodular sclerosis (71.2%), followed by mixed cellularity (22.8%), lymphocyte rich (3.8%) and lymphocyte depleted (1.9%). Median follow up time was 45 months (range 0-136). The 10-year overall survival for the favorable group (n=329) was 77% (95% CI: 71.1-88.8) versus 68% for the unfavorable group (n=144) and 42% for the advanced group (372) (p<0.001).

There was no statistical difference in survival between stage I (n=170), and stage II (n=385) disease (p=0.99). Treatment modalities were then compared for the favorable risk group alone. Those who received chemotherapy alone (n=145) were compared to those who received combined chemotherapy and radiation (n=148) as their primary therapy. The 10-year overall survival for the cohort receiving chemotherapy and radiation was 87% compared to 75% for those receiving only chemotherapy (p<0.001) (Figure 1). When adjusted by multivariate analysis for risk factors affecting 10 year survival of the favorable cohort, only age <50 and the treatment modality were independently associated with a statistically significant difference in overall survival (HR of 0.11 (p<0.001) and 3.94 (p=0.001), respectively).

Figure 1.

Summary/Conclusions: Our large data cohort shows the presence of B symptoms to be prognostic than the number of nodal regions involved for early stage disease. Although the use of radiation as part of initial therapy for early stage disease might have increase long term toxicity, it continued to provide superior survival at 10 years.
THE IMPACT OF TREATMENT WITH BRENTUXIMAB VEDOTIN ON OVERALL SURVIVAL OF PATIENTS WITH HODGKIN LYMPHOMA RELAPSED AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: A NATIONWIDE POPULATION BASED ANALYSIS


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Background: Patients with Hodgkin Lymphoma (HL) who relapse after autologous Cell Transplantation (auto-SCT) have a dismal prognosis. Advanced disease stage, presence of B-symptoms, extranodal involvement at the time of relapse and duration of remission of less than 12 months are parameters associated with decreased overall survival (OS). Brentuximab Vedotin (BV), an anti-CD30 monoclonal antibody conjugated to a microtubule-disrupting agent has shown clinical efficacy in HL. Although in the setting of post-auto-SCT relapse, BV produces an overall response rate of approximately 75% with a median progression free survival (PFS) of 9 months, the impact of BV on OS has not been addressed in previously published studies.

Aims: To examine the impact of treatment with BV on OS of patients with HL relapsed after auto-SCT.

Methods: Data for patients with HL who underwent auto-SCT in Greece during the last 20 years were collected. Study group consisted of 214 patients who experienced post-auto-SCT relapse. In order to examine the impact of BV on OS, patients were divided in 2 cohorts depending of the date of BV availability in Greece (January 2013). Cohort 1 consisted of 178 patients who relapsed before January/2013, while Cohort 2 consisted of 36 patients relapsed after BV became available. Patient’s characteristics are shown in Table 1.

Table 1. Patients characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26 (14-64)</td>
<td>24 (19-69)</td>
<td>p=0.53</td>
</tr>
<tr>
<td>Sex: male (yes vs no)</td>
<td>0.615</td>
<td>0.735</td>
<td>p=0.62</td>
</tr>
<tr>
<td>B-symptoms (yes vs no)</td>
<td>0.015</td>
<td>0.002</td>
<td>p=0.001</td>
</tr>
<tr>
<td>Stage (I-II vs III-IV)</td>
<td>0.703</td>
<td>0.822</td>
<td>p=0.35</td>
</tr>
<tr>
<td>Extralobar disease</td>
<td>0.381</td>
<td>0.267</td>
<td>p=0.20</td>
</tr>
<tr>
<td>Time from 1st CT to relapse (days)</td>
<td>119 ± 72</td>
<td>60 ± 30</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Previous allogeneic SCT</td>
<td>0.420</td>
<td>0.438</td>
<td>p=0.80</td>
</tr>
<tr>
<td>Advanced disease stage (yes vs no)</td>
<td>0.128</td>
<td>0.213</td>
<td>p=0.63</td>
</tr>
<tr>
<td>BV after relapse</td>
<td>0.012</td>
<td>0.002</td>
<td>p=0.001</td>
</tr>
<tr>
<td>BV vs auto-SCT relapse</td>
<td>0.013</td>
<td>0.002</td>
<td>p=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 allologeneic SCT or treatment with immune checkpoint inhibitors (IC-inhibitors).

Results: In multivariate analysis the following variables were statistically associated with OS: 1) The presence of B-symptoms [HR=2.07, (95% CI, 1.39-3.07), p=0.001] and 2) Relapse in less than 12 months after auto-SCT [HR=4.76, (95% CI, 3.35-5.00), p=0.001] were associated with decreased OS, while 3) Response after 1st salvage [HR=0.46, (95% CI, 0.31-0.68), p=0.001] and 4) BV availability [HR=0.36, (95% CI, 0.16-0.79), p=0.011] were associated with increased OS (Figure 1). Similar results were obtained when analysis was performed without censoring patients at the time of allo-SCT or treatment with IC-inhibitors (data not shown).

Summary/Conclusions: Patients in Cohort 2 survived longer even when censored for allo-SCT or treatment with IC-inhibitors. All patients in Cohort 2 treated with BV while only 18% of patients in Cohort 1 received treatment with BV. The results of our study strongly suggest that BV improves OS in patients with HL relapsed after auto-SCT. To our knowledge this is the first study showing an OS advantage of treatment with BV.
tion was defined as imaging at or before week 12 of treatment, whereas late radiological evaluation was performed at or after week 16. Response evaluation was performed according to the Lugano Classification and its update regarding immunomodulatory therapy.

Results: Between 06/2015-11/2016, 87 patients were enrolled in a name-based program in Turkey. Two, 19, and 3 patients who had not yet received nivolumab, had not reach the time for early radiological evaluation, and who died before any radiological evaluation were excluded from the analysis. Thus, 63 patients from 23 centers were retrospectively analyzed. Median follow-up was 6 months, median age was 29 (18-75) and patients had a median 5 (2-11) previous lines of therapy. 44 patients (70%) had been treated by stem cell transplantation (SCT) and 45 (76%) patients had been treated by BV. The ORR was 66% with 15 CR (95%CI 0.020-0.28, CR 26%, PR 42, SD 12%, PD 20%) among 59 patients evaluated in 12 weeks of nivolumab treatment. The ORR was 67% with 9 (24%) patients with CR after 16 weeks of treatment (95%CI 0.004-0.26, CR 24%, PR 43%, SD 6%, PD 27%). Estimated OS was 95% (95%CI 0.90-0.98) 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).

Figure 1.

Summary/Conclusions: In conclusion PD-1 blockers are new options to meet the unmet need in patients with chHL refractory to BV treatment, and possibly a bridge for these patients before transplantation.

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GENOTYPING OF HODGKIN LYMPHOMA ON THE LIQUID BIOPSY

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Background: In classical Hodgkin lymphoma (cHL) the low representation (1-5%) of Reed-Sternberg cells (RS) challenged tumor genotyping on the diagnostic tissue biopsy. Consistently, the mutational profile of newly diagnosed cHL is poorly characterized, and the genetics of refractory disease is completely unknown. Cell free DNA (cfDNA) is shed into the blood by tumor cells under-going apoptosis and can be used as source of tumor DNA for the identification of somatic mutations. In addition cfDNA is representative of the entire tumor heterogeneity, thus allowing the identification of mutations from tumor cells residing in non-biopsied sites.

Aims: This study aims: i) at providing the evidence that the mutational profile of cHL can be tracked by using plasma cfDNA; and ii) at characterizing the genetics of newly diagnosed cHL and, for comparative purposes, of refractory cHL.

Methods: The study includes 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 >200x in >80% of the target region in all samples, which allowed a sensitivity of 3x10⁻3. The somatic function of VarScan2 was used to call non-synonymous somatic mutations, and a stringent bioinformatic pipeline was applied to suppress the background noise and to filter out sequencing errors.

Results: In newly diagnosed cHL, genotyping of plasma cfDNA identified non-synonymous somatic mutations in STAT6 (43%), TNAIP3 (43%), ITPKB (32%) B2M (21%), GNA13 (14%), CIITA (7%), XPO1 (7%) and CD58 (4%) among the most recurrently affected genes (Figure 1A-B). In refractory cHL patients, genotyping of plasma cfDNA identified non-synonymous somatic mutations in ITPKB (44%), TNAIP3 (33%), KMT2D (33%), B2M (33%), GNA13 (33%), XPO1 (22%), TET2 (22%), IKBKB (22%), BIRC3 (22%) and STAT6 (22%) among the most recurrently affected genes. Mutations of KMT2D (33%) and TET2 (22%) were enriched in refractory chHL 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).

Figure 1.

Summary/Conclusions: This study provides the evidence that cfDNA can be genotyped using plasma cfDNA as source of tumor DNA, pointed to a non-overlapping genotype between newly diagnosed and refractory cases, and identified ITPKB as a new gene specifically involved in ~30-50% of cHL patients.
FDG PET-CT MAYBE A USEFUL TOOL TO IDENTIFY DOXORUBICIN INDUCED CARDIOTOXICITY IN HODGKIN LYMPHOMA

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Background: Doxorubicin (DXR) induced cardiotoxicity is related to several mechanisms, including interference of mitochondrial respiratory chain and acceleration of glycolysis. We previously reported that this treatment may enhance myocardial FDG uptake.

Aims: The present study aimed to verify whether this metabolic response on serial PET/CT imaging can predict myocardial function, non-invasively evaluated by follow-up echocardiography (ECHO).

Methods: 18F-FDG PET/CT of 25 patients affected by Hodgkin Disease (HD), treated following ABVD scheme were analyzed. Inclusion criteria were: 1) availability of 4 consecutive PET/CT scan for staging (PET1), interim (PET2), post-therapy (PET3) and six months follow-up evaluation (PET4); 2) full remission 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 without any progressive increase. LV-SUV progressively increased from PET1 to PET4 in 6 patients (24%, 2 females, mean age 38±17, termed “increasers”) being 1.34±0.9, 3.34±2.6, 4.32±2.8 and 4.43±1.5 respectively. In the remaining 19 patients (76%, 7 females, 36±14), FDG uptake showed a largely variable response without any progressive increase. Accordingly, the ratio between PET4 and PET1 LV-SUV in the two subgroups was 3.85±0.8 and 1.06±0.4, respectively (p=0.001). Up to six months after therapy discontinuation, none of the 22 patients showed signs or symptoms potentially related to DXR cardiotoxicity. However, late follow-up ECHO detected the appearance of first-degree diastolic impairment with respect to baseline in 9 of the 25 examined patients (36%, 4 females, mean age 36±18). This finding occurred in 5/6 “increasers” (83%) and in only 4/19 non-increasers (21%) (p=0.001).

Summary/Conclusions: The present data indicate that DXR related myocardial damage can be preceded by an enhanced glucose uptake. 18F-FDG PET/CT imaging might represent a useful tool to identify high-risk patients and to implement personalized program to monitor and prevent DXR-induced cardiotoxicity.

Iron metabolism, deficiency and overload

ELEVATED SYSTEMIC HEME AND IRON LEVELS AS RISK FACTORS FOR VASCULAR DYSFUNCTION ANDATHEROSCLEROSIS: EVIDENCE FROM B-THALASSEMIA 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 those mice.

Aims: Here we aimed at evaluating the clinical relevance of these findings and mechanisms, including interference of mitochondrial respiratory chain and acceleration of glycolysis. We previously reported that this treatment may enhance myocardial FDG uptake.

Elevated 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 increased 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 hemoglobin, heme and iron in the pathogenesis of vascular dysfunction in β-thalassemia and hemochromatosis and suggest a pro-atherosclerotic role for these molecules. These findings are relevant, on one side, for cardiovascular diseases and vasculopathy, when iron parameters are altered, and on the other, for iron overload disorders, where premature atherosclerosis might develop. Finally, our data highlight the key protective role of heme/iron scavengers and support the potential therapeutic benefit of chelation therapy to counteract heme/iron-driven vascular toxicity and atherosclerosis in hemolytic and iron-overload conditions.
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 Datavarse (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 CT claims (base year), and ≤16 months of prior use of DFX DT pre-index. Medication possession ratio (MPR) (percentage of time with access to medication) was computed for DFX DT during the “DFX DT period” (from earliest DFX CT 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 CT 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 sign-rank test for continuous data and McNemar’s test for dichotomized data.

Results: Of the 606 eligible pts, 56% were female, 64% were <35 years old, and 42% had transfusions during the baseline period. The median durations of the DFX DT and DFX FCT periods were 350.9 days and 290.2 days, respectively. Compared with adherence to DFX DT, adherence to FCT was significantly improved across all measures. Mean MPR of DFX CT 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 CT vs DFX DT was 0.83 vs 0.71 (p<0.001); 50.0% pts had mean 3-month PDC ≥0.8 during the DFX FCT period compared to 34.5% during the DFX DT period (p<0.001). The proportion of pts with 3-month persistence to DFX CT 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 higher adherence and persistence to 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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MEDIATION BY PATIENT-REPORTED OUTCOMES ON THE ASSOCIATION BETWEEN FILM-COATED VERSUS DISPERSIBLE FORMULATIONS OF DEFERASIROX AND SERUM FERRITIN REDUCTION: A POST HOC ANALYSIS OF THE ECLIPSE TRIAL

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Background: The ECLIPSE clinical trial (NCT02125877) demonstrated that a new film-coated tablet (FCT) formulation of deferasirox (DFX) had a similar safety profile, with fewer patients (pts) experiencing severe gastrointestinal (GI)-related adverse events (AEs), and more favorable patient-reported outcomes (PROs) compared to the dispersible tablet (DT). However, the PROs of FCT pts showed better adherence, satisfaction, palatability and fewer concerns about iron chelation therapy (ICT). ICT pts had a higher median absolute reduction in serum ferritin (SF) from baseline to the end of treatment (tx) (-350.0 mg/L). The improvement with DFX-FCT suggests that ICT can be further augmented with this formulation. This real-world study complements the ECLIPSE trial results and supports previous evidence of improved adherence to DFX FCT.

Aims: To examine the proportion of the association between tx with FCT vs DT and SF reduction from baseline that is mediated through PROs, in a post hoc analysis.

Methods: The ECLIPSE trial was a phase II, randomized, open-label study in which 173 pts with transfusion-dependent thalassemia or myelodysplastic syndrome were randomized to receive DFX FCT (N=87) or DT (N=86) at average planned doses of 30.7 mg/kg/day for 6 months and SF reduction from baseline (±5% of baseline value). PROs were assessed at weeks 2, 3, 13, and 24 (end of tx) using the Palatability and Modified Satisfaction with Iron Chelation Therapy (mSICIT) questionnaires, the latter assessing 3 domain scores: adherence, satisfaction, and concerns. Frequency of GI-related AEs was assessed during the tx period. Mediation analysis, i.e., comparing a model adjusted for hypothesized mediators to an unadjusted model (Lin DY, et al Stat Med. 1997), was used to compute proportion mediated (PM). PM quantifies how much of the association between tx with DFX FCT versus DT and SF reduction from baseline is operationalized through pt-reported adherence score, oth PRO scores, and frequency of GI-related AEs during tx. The analysis was adjusted for confounders including age, sex, race, underlying hematological disease, prior use of DFX DT, baseline level of iron overload severity, average planned dose, and number of blood transfusions on tx. Sensitivity analyses were conducted in subgroups of pts who had prior use of DFX DT (DT naïve), had thalassemia, and were DT non-naïve pts with thalassemia.

Results: The association between tx with DFX FCT versus DT and SF reduction was substantially mediated by pt-reported adherence (PM=66.6%, p<0.01). Pt-reported adherence, along with pt-reported satisfaction, concerns, and palatability scores, and frequency of GI-related AEs together mediated 76.7% of the association (p=0.02). The proportion mediated was increased in the DT non-naïve subgroup (PM=92.7%, p<0.03). Similarly, an increased PM was seen in the subgroup of DT non-naïve pts with thalassemia compared to all thalassemia pts (PM=70.1%, p<0.08 vs PM=60.0, p<0.12, respect).

Summary/Conclusions: Better PROs, especially increased pt-reported adherence, due to improved attributes associated with DFX FCT are significant intermediates of the association between tx with DFX FCT versus DT and SF reduction from baseline. The proportion mediated was increased in pts with prior DT exposure, suggesting their enhanced appreciation for DFX FCT over DT.

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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) R2-MRI together with a core laboratory service has been validated in routine clinical practice, methods relying on high-field magnetic resonance imaging (MRI) and/or on more time consuming methods for acquisition protocols and data analysis have not yet been validated in this way.

Aims: To determine the limits of agreement between measurements of LIC by a widely available T2*/R2* MRI method and a reference standard SDPA R2-MRI method in a routine clinical practice setting.

Methods: 119 thalassemia patients (N=100) referred by the National Institute of Hematology and Blood Transfusion, Hanoi, Vietnam for routine LIC measurement by MRI were prospectively recruited with informed consent. Patients were randomised to be scanned in either a Philips Ingenia or a Siemens Avanto 1.5T scanner. The LIC of each patient was measured twice, once by a T2*/R2* technique using our own customized software and protocols (Iron Health Calculator: http://www.ironcalculator.com) and once by SDPA R2-MRI using a quality controlled core laboratory data analysis software (FermScan®). Analyses using the T2*/R2* data analysis method were blinded from the SDPA R2-MRI results and vice versa. Reported data were analysed using the statistical methods of Bland and Altman.

Results: A plot of the T2*/R2* LIC against the SDPA R2-MRI LIC (Figure 1) shows the vast majority of the data falling below the line of equivalence indicating that the T2*/R2* method is understimating the LIC relative to the SDPA R2-MRI validated reference standard. The geometric mean ratio of T2*/R2* LIC to SDPA R2-MRI LIC was 0.44 (95% CI 0.36 – 0.55) indicating severe underestimation of LIC by the T2*/R2* method. The geometric mean ratios of the two LIC measurements were significantly different for the two scanners (0.28 for Philips and 0.68 for Siemens, p <0.0001) indicating that the bias of the T2*/R2* method against the reference standard is not universal but is dependent on both/either scanner type and/or data acquisition method. Bland Altman analysis indicates that 95% of pairs of measurements are predicted to have ratios between 3.73 and 0.05 indicating a very large random variability between the T2*/R2* method and the reference standard. The performance of the T2*/R2* method against the SDPA R2-MRI LIC values above the clinical relevant thresholds of 7 and 15 mg Fe/g dw is characterized in the Table 1 showing positive predictive values (PPVs) and negative predictive values (NPVs) together with their 95% CIs.

Table 1.
Mean (SD) duration of deferasirox exposure in group A was 7.5 (1.7) years; mean daily deferasirox dose was 540 mg.

In both subgroups analyzed, mean Scr was within normal limits and remained stable over time during the retrospective period (Figure 1). Analysis in adults showed mean Scr values were stable over time. As expected in growing children who are gaining height and weight, pediatric mean Scr absolute values increased from baseline in proportion with an almost linear increase in muscle mass over time.

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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 reported a retrospective analysis of patients with transfusion-dependent anemia during a decade of deferasirox treatment indicating stable and a lack of any progressive worsening of renal function (Origa R et al. Blood 2016).

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

Methods: Italian patients with β thalassemia, SCD, myelodysplasic 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 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.
Methods: A cohort of 25 well characterized patients was analyzed. Eighteen were initially referred to our center for unexplained hypereferritinemia (HF), two for proven iron overload (IO) by MRI, 2 for chronic hemolysis and 3 for aregenerative anemia. A set of phototypic 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 subclinical iron overload was performed. Phototypic investigations failed to clearly identify the cause of the disorder. Therefore, each patient was tested for a panel of 32 genes involved either in iron homeostasis or hereditary anemias, using NGS. Libraries were obtained using the Custom SureSelectQXT Target Enrichment system (Agilent, Santa Clara Ca USA) and sequenced on a MiSeq platform (illumina, San Diego, Ca, USA). Each deleterious variation was independently checked using conventional Sanger sequencing. Written informed consent was obtained from all the patients for NGS genetic analyses.

Results: Initial phototypic reassessment allowing classifying the patients into 5 different groups: 1/ isolated hypereferritinemia (n=11); 2/ HF and IO (MRI >90 µmole/g dry weight) (n=17); 3/ hereditary anemia (HA) without IO (n=2); 4/ HA and IO (n=2); 5/ aregenerative anemia with IO (n=3). Among patients with an initial diagnosis of iron disorder, the reticulocyte count identified 2 undiagnosed chronic fully compensated hemolysis. Systematic screening using the gene panel identified a total of 14 sequence variations of clinical significance in 9 different genes and 9 patients. An isolated mutation was found in 7 and 2 patients with an initial diagnosis of iron or of red cell disorder respectively. A combined anomaly of red cell and iron genes was identified in 3 patients who displayed IO and compensated hemolysis or anemia. Digemism 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 phototypic 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 on 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.

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CHANGES IN LIVER IRON CONCENTRATION R2 MRI MEASUREMENT ACROSS DIFFERENT CHELATION REGIMENS IN PATIENTS WITH HEMATOLOGICAL DISORDERS: REAL-LIFE EXPERIENCE FROM LICNET

Aims: To report a real-life experience of liver iron chelation in different hematological disorders, including iron-overload in thalassemia, sickle cell disease, and iron-refractory anemia with reticulocytosis.

Methods: A total of 130 patients were evaluated in this analysis, with a median age of 35 years (range: 6–78) and including 60 (46.2%) men. The median duration (range) between the first and second MRI was 483 days (184-1076) and was comparable between iron chelation regimens. Median pre-transfusion hemoglobin level and blood requirement were similar at both MRIs. The median change in LIC (range) in mg Fe/g dw was not significant in patients receiving DFO (n=20, median change -1.9, p=0.55), DFO+DFP (n=52, median change -0.5, p= 0.155), DFO+DFP (n=10, median change -2.2, p=0.074), or other combinations (n=7, median change -1.3, p=1.000), while it decreased significantly on DFO monotherapy (n=32, median change -1.4,p=0.002). Among oral chelators, DFX showed to be more effective, during the period of the study, in stabilizing iron body burden in 65.4% patients even if they had baseline LIC values <7mg Fe/g dw (median 4.0 mg Fe/g dw) and with similar response as combined treatment DFO+DFP (Figure 1).

Summary/Conclusions: This cohort study suggests that stabilization of LIC is achievable, during a median of 483 days, with different iron chelation regimens in real life experience, with considerable proportions of patients shifting to more favourable LIC categories. Therefore, the periodic determination of LIC by MRI has to be strongly recommended for management and prevention of iron overload and subsequent complications in haematological disorders.

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

Aims: To evaluate the effect of maternal IDA on auditory neural myelination in full-term neonates using the auditory brainstem evoked response (ABR) as noninvasive neurophysiological assessment tool.

Methods: This prospective case-control study was conducted at Ain Shams University Hospitals and included 100 neonates. Out of 223 pregnant women, 50 were diagnosed as having IDA anemia and 50 healthy mothers were enrolled as a control group. Neonates were studied focusing on anthropometric measures, hematologic profiles and iron status. Auditory brainstem response (ABR) was recorded using evoked response audiometry technique (FernScan® Resonare Health Limited, Claremont, WA, Australia) approved by the FDA. The LICNET (Liver Iron Content NETwork) was established by Foundation Franco e Piera Cutino di Palermo and is addressed to diagnostics of liver iron overload by R2 MRI in subjects with hemochromatosis in haematological disorders. The LICNET protocol was approved on December 4, 2012 by Ethics Committee. Baseline data from LICNET were, recently, published (Vitrano et al., 2016, EJH).

Aims: The aim of this study was to evaluate longitudinal changes in LIC measurements across different iron chelation regimens in a real-life cohort of patients with transfusional iron overload included in LICNET.

Results: A total of 130 patients were evaluated in this analysis, with a median age of 35 years (range: 6-78) and including 60 (46.2%) men. The underlying diagnoses were regularly transfused thalassemia major (n=86, 66.2%), thalassemia Intermedia (n=33, 25.4%), sickle cell disease (n=6, 4.6%), myelodysplastic syndrome (n=5, 2.3%), and Diamond-Blackfan anemia (n=2, 1.5%). The median duration (range) between the first and second MRI was 43 days (184-1076) and was comparable between iron chelation regimens. Median pre-transfusion hemoglobin level and blood requirement were similar at both MRIs. The median change in LIC (range) in mg Fe/g dw was not significant in patients receiving DFO (n=20, median change -1.9, p=0.55), DFO+DFP (n=52, median change -0.5, p= 0.155), DFO+DFP (n=10, median change -2.2, p=0.074), or other combinations (n=7, median change -1.3, p=1.000), while it decreased significantly on DFO monotherapy (n=32, median change -1.4,p=0.002). Among oral chelators, DFX showed to be more effective, during the period of the study, in stabilizing iron body burden in 65.4% patients even if they had baseline LIC values <7mg Fe/g dw (median 4.0 mg Fe/g dw) and with similar response as combined treatment DFO+DFP (Figure 1).

Figure 1.

Summary/Conclusions: This cohort study suggests that stabilization of LIC is achievable, during a median of 483 days, with different iron chelation regimens in real life experience, with considerable proportions of patients shifting to more favourable LIC categories. Therefore, the periodic determination of LIC by MRI has to be strongly recommended for management and prevention of iron overload and subsequent complications in haematological disorders.
Table 1. ROC Curve Analysis.

<table>
<thead>
<tr>
<th>LIC Threshold (mg Fe/g)</th>
<th>SF (mg/L)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 1</td>
<td>1270</td>
<td>0.68 (0.51 - 0.85)</td>
<td>0.90 (0.83 - 0.93)</td>
<td>0.80 (0.70 - 0.93)</td>
</tr>
<tr>
<td>1 - 12</td>
<td>1270</td>
<td>1.00 (0.80 - 1.00)</td>
<td>0.90 (0.83 - 0.93)</td>
<td>0.80 (0.70 - 0.93)</td>
</tr>
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Figure 1.
rtin and transferrin, with minimal risk of release of large amounts of ionic iron in the serum.

Aims: To compare safety and efficacy of Ferric Carboxymaltose (FCM) with Iron Sucrose complex (ISC) regarding improvement in haematological parameters and side effects in women with iron deficiency anaemia (IDA).

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

Results: Two FCM infusions vs five ISC infusions were required. On day 28 Hb increment ≥3g/dl was seen in 63.33% and MCV>80FL seen in 100% of FCM group vs 0% and 43.33% in ISC group. Significant improvement in RBC indices & retic count was seen in FCM group. Earlier and significant improvement in fatigability & dyspnoea on exertion was observed in FCM group. Both groups had similar safety profile except for thrombophlebitis was observed in 8.67% FCM group vs 50.00% ISC group.

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

Lymphoma biology

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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 × 10⁻12) and for nodular sclerosis HL (NSHL) at 3q28 (rs4459895, P=9.49 × 10⁻11). 5q31 (rs9298977, AHI1, P=4.62 × 10⁻10), 10p14 (rs3781093, GATA3, P=9.49 × 10⁻13), 13p34 (rs112988813, UFP3A, P=4.58 × 10⁻10) and 16p13 (rs34972832, CLEC16A, P=1.29 × 10⁻9). Additionally, independent loci within the HLA region were observed for NSHL (rs2689081, HLA-DPB1*03:01, Val86 in HLA-DRB1) and mixed cellularity HL (rs1633096, rs13196329, Val86 in HLA-DRB1). Expression quantitative trait loci were observed in lymphoblastoid cells from 825 individuals at 6q22 (AHI1, P_SMR<0.001), 10p14 (GATA3, P_SMR=4.70×10⁻8) across new and established risk loci confirmed a significant enrichment of DNA hypersensitivity in GM12878 cells (P=1.20 × 10⁻3) as well as regulatory elements in primary B-cells (P=6.0 × 10⁻5) and 825 individuals (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 REL (nuclear factor NF-kappa-B p65), EBF1 (early B-cell factor 1), RUNX3 (runt-related transcription factor 3) and BATE (histone deacetylase 4). 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 MANTLE CELL LYMPHOMA

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Background: Mantle Cell lymphoma (MCL) is one of the most aggressive...
phoid neoplasms characterized by highly infiltrated tumor cells in lymphoid tissues and extranodal 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 first generated a stable transduced SOX11-silenced MCL cell line and compared it with a scrambled control MCL cell line. Then we infected MCL cell lines with SOX11-1 positive lentiviral particles carrying shRNAs 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 influence of SOX11 overexpression in B-cell lymphomas and in the transduction of SOX11 in primary xenograft models. We also analyzed the distribution of SOX11-positive and SOX11-negative cells in spleens, lymph nodes, bone marrow, and peripheral blood for patients with CNS lymphoma our main aim is to assess the role of XPO-1 inhibition in intracerebral xenograft models.

**Results:** In the si mice model, we observed that SOX11 silencing reduced tumor growth compared to SOX11-positive control tumors. We analyzed the gene expression profiling of these xenograft tumors and of SOX11-positive and negative primary cases and we observed that different microenvironment-related signatures were enriched in SOX11-positive compared with SOX11-negative cells, as angiogenesis, migration and stromal stimulation. By ChIP-chip analysis, we found that AICDA is highly expressed in SOX11-repressed MCL cell lines and in the transduction of MCL xenograft model. We also found that SOX11 promotes angiogenesis in MCL cells through PDGFA regulation, promoting tumor growth and vasculature. Inhibition of PDGFA on endothelial cells of SCID mice (n=4) and MCL, and the transduction of SOX11-negative MCL (n=6), displayed a reduced tumor burden in xenograft model of PCNSL by stereotactic injection of OCI-Ly10 (ABC, MYD88 and CD79b) cells expressing luciferase into the cerebral parenchyma of SCID mice. Our results demonstrated that AICDA acts as a methylome modifier in GC-DLBCL, being most of them related to activated B-cell type (ABC-DLBCL). PCNSL is associated with poor prognosis, particularly because of the difficulty for drugs to cross the blood brain barrier. High dose methotrexate is the most effective treatment, but relapse is very common and salvage treatment options are scarce. Also, in patients with systemic lymphoma, the secondary infiltration of the CNS is a fatal event, with a global overall survival of less than six months. Therefore, the development of new drugs with ability to penetrate the CNS is highly needed. Selinexor (KPT-330) is a Selective Inhibitor of Nuclear Export (SINE) that inactivates XPO-1 protein and induces anti-tumor effects mainly due to forcing nuclear retention and activation of tumor suppressors. Selinexor has shown excellent brain penetration and promising results in pre-clinical models of glioblastoma and can inhibit both BCR and NF-kB signaling in malignant B-cells.

**Aims:** In order to provide a pre-clinical rationale for the design of new therapies for patients with CNS lymphoma our main aim is to assess the role of XPO-1 inhibition in intracerebral xenograft murine models.

**Methods:** We in vitro tested the sensitivity of DLBCL cell lines to selinexor and ibritinib by MTS and AnnexinV/PI assay. We established an orthotopic xenograft model of PCNSL by stereotactic injection of OCI-Ly10 (ABC, MYD88 and CD79b) cells expressing luciferase into the cerebral parenchyma of SCID mice. Our results demonstrated that AICDA acts as a methylome modifier in GC-DLBCL, being most of them related to activated B-cell type (ABC-DLBCL). PCNSL is associated with poor prognosis, particularly because of the difficulty for drugs to cross the blood brain barrier. High dose methotrexate is the most effective treatment, but relapse is very common and salvage treatment options are scarce. Also, in patients with systemic lymphoma, the secondary infiltration of the CNS is a fatal event, with a global overall survival of less than six months. Therefore, the development of new drugs with ability to penetrate the CNS is highly needed. Selinexor (KPT-330) is a Selective Inhibitor of Nuclear Export (SINE) that inactivates XPO-1 protein and induces anti-tumor effects mainly due to forcing nuclear retention and activation of tumor suppressors. Selinexor has shown excellent brain penetration and promising results in pre-clinical models of glioblastoma and can inhibit both BCR and NF-kB signaling in malignant B-cells.

**Aims:** The goal of our study was to identify the spectrum of genes regulated by SOX11 in malignant lymphoid cells and provide insights on how the constitutive overexpression of SOX11 may contribute to the oncogenic development of MCL.

**Methods:** We first generated a stable transduced SOX11-silenced MCL cell line and compared it with a scrambled control MCL cell line. Then we infected MCL cell lines with SOX11-1 positive lentiviral particles carrying shRNAs 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 influence of SOX11 overexpression in B-cell lymphomas and in the transduction of SOX11 in primary xenograft models. We also analyzed the distribution of SOX11-positive and SOX11-negative cells in spleens, lymph nodes, bone marrow, and peripheral blood.

**Results:** In the si mice model, we observed that SOX11 silencing reduced tumor growth compared to SOX11-positive control tumors. We analyzed the gene expression profiling of these xenograft tumors and of SOX11-positive and negative primary cases and we observed that different microenvironment-related signatures were enriched in SOX11-positive compared with SOX11-negative cells, as angiogenesis, migration and stromal stimulation. By ChIP-chip analysis, we found that AICDA is highly expressed in SOX11-repressed MCL cell lines and in the transduction of SOX11 in primary xenograft models. We also found that SOX11 promotes angiogenesis in MCL cells through PDGFA regulation, promoting tumor growth and vasculature. Inhibition of PDGFA on endothelial cells of SCID mice (n=4) and MCL, and the transduction of SOX11-negative MCL cell lines, displayed a reduced tumor burden in xenograft model of PCNSL by stereotactic injection of OCI-Ly10 (ABC, MYD88 and CD79b) cells expressing luciferase into the cerebral parenchyma of SCID mice. Our results demonstrated that AICDA acts as a methylome modifier in GC-DLBCL, being most of them related to activated B-cell type (ABC-DLBCL). PCNSL is associated with poor prognosis, particularly because of the difficulty for drugs to cross the blood brain barrier. High dose methotrexate is the most effective treatment, but relapse is very common and salvage treatment options are scarce. Also, in patients with systemic lymphoma, the secondary infiltration of the CNS is a fatal event, with a global overall survival of less than six months. Therefore, the development of new drugs with ability to penetrate the CNS is highly needed. Selinexor (KPT-330) is a Selective Inhibitor of Nuclear Export (SINE) that inactivates XPO-1 protein and induces anti-tumor effects mainly due to forcing nuclear retention and activation of tumor suppressors. Selinexor has shown excellent brain penetration and promising results in pre-clinical models of glioblastoma and can inhibit both BCR and NF-kB signaling in malignant B-cells.
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 lived significantly longer increasing by about 60% (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 such, is expected to include many heterogeneous tumors. In fact, recent genetic studies have suggested that a subset of PTCL-NOS is closely related to angioimmunoblastic T-cell lymphoma (AITL); both lymphoma types show follicular helper T-cell (TFH) phenotypes and share mutational targets in common, such as ROHA, 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 ROHA (28%), TP53 (18%), KMT2C (13%), IDH2 (11%), and PLCG1(11%). Frequently altered genes included signal transduction molecules (such as ROHA, 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, YTHFD2, and LRPIB, which were frequently targeted by nonsense and frameshift mutations distributed throughout the entire genes. Among these, PDCD1 encodes PD-1, which transmits an inhibitory signal from PD-L1 and PD-L2 ligands, and therefore loss of function of this gene is predicted to enable malignant T-cells to escape from the negative signaling. By contrast, recurrent mutations in YTHFD2 and LRPIB mutations in T-cell lymphoma genesis is unexpected. These genes encode a repressor protein of N6-methyladenosine (YTHFD2), and a member of the low density lipoprotein receptor family (LRPIB). Although the function of these genes in T-cells are unknown, our findings suggest their unresolved roles, whose dysfunction may lead to malignant T-cell proliferation. Finally, we investigated the co-occurrence between frequently mutated genes in PTCL-NOS. In accordance with previous observation, mutations characteristic of TFH lymphomas (TET2, ROHA, 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 METHYLMOD 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 dessect 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 about 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 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 of DNA methylation signals in B cell tumors revealed that samples with less than 55% tumor cell content could not be accurately purified. This strategy reduced the initial 1,044 tumor samples to 866. An unsupervised principal component analysis of in silico purified data revealed that each type of B-cell neoplasm clusters separately, ALLs clustered closer to precursor B cells, CLL and MCL closer to mature B cells and both DLBCL and MM showed the largest deviation from normal B cells. We then performed a differential analysis of 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 for 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.
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, in a subset of patients with diffuse large B-cell lymphoma. Proton magnetic resonance imaging of mito- tosine dioxide 2 in up to 70% of AITL and other TFH lymphoma (a sub- group 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). GNEFs are thought to have diverse roles in cell migratory signaling, because it does not bind GTP. Therefore, it has remained unknown how G17V RHOA is involved in lymphomagenesis. VAV1 serves as an impor- tant mediator of T-cell receptor (TCR) signaling pathway through its GEF- dependent and -independent function. VAV1 activation is tightly regulated by specific binding partner proteins of the G17V RHOA mutant. 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 sig- naling.

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

Methods: Proteomic screening was performed to identify G17V RHOA-spe- cific binding partner proteins. Binding was validated by co-immunoprecipitation of G17V RHOA and the candidate partners. Simultaneously, RNA sequencing was performed for 9 PTCL samples, including 6 AITL and 3 PTCL-NOS. Target- ed 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 constructs.

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 acidic region (c.G516_C520del:p.173_C177del and c.C494_C504del: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.C1844T: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 treatment. The levels of IL-2 mRNA were higher in Jurkat cells expressing either G17V RHOA or VAV1-STAP2 than those with their WT or mock. Finally, phospho-VAV1 was co- stained with PD-1, a TFH marker, in 7 out of 10 PTCL samples with RHOA or VAV1 mutations.

Summary/Conclusions: The G17V RHOA and VAV1 mutants both intensify the TCR pathway through hyper-phosphorylation of Vav1. Our data suggest that the RHOA-VAV1 axis in AITL/TFH lymphoma may contribute to their clinical features and stand as a possible new therapeutic target.
formation using a bespoke bioinformatic pipeline based on TargetScan prediction algorithm in order to identify mutations in putative miRNA binding sites. Once identified, in order to validate them and test their recurrence in an extended cohort (60 samples from 31 FL patients who underwent transformation plus 21 samples of non-transformed FL patients) we designed an Ampliseq (Ion Torrent, Life Technologies) NGS custom panel. Finally, we selected a number of variants for assessing the variant effect on the miRNA: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 prioritised 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).

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.

**Figure 1.**

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

**P304**

**CLINICAL IMPACT OF TP53 AND KMT2D MUTATIONS IN MCL RECEIVING HIGH-DOSE THERAPY AND AUTOLOGOUS TRANSPLANTATION: UPDATED RESULTS FROM THE FONDAZIONE ITALIANA LINFOMI MCL0208 PHASE III TRIAL**

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**Background:** Within the landscape of mutated genes in mantle cell lymphoma (MCL), only TP53 disruption has been so far associated with outcome.

**Aims:** Here we present the clinical update of the deep sequencing MCL gene panel analysis in the prospective FIL-MCL0208 phase III trial (NCT02334313), high-dose immunochemotherapy followed by autologous transplantation for untreated, advanced stage <65 years MCL based on the data from the second interim analysis.

**Methods:** A targeted resequencing gene panel, including coding exons and splice sites of the ATM, BIRC3, CCND1, KMT2D, TP53, TRAF2, WHSC1, and NOTCH1 genes was analyzed in tumor DNA from baseline bone marrow CD19+ purified MCL cells and, to filter out polymorphisms, in the paired normal genomic DNA (55% of cases) using a TrueSeq Custom Amplicon target enrichment system followed by deep next generation sequencing (Illumina, median depth of coverage 235x). Variants represented in >10% of the alleles were called with VarScan2 with the somatic function when the paired germline DNA was available. For patients lacking germline DNA, a bioinformatics pipeline including a number of stringent filters was applied to protect against the misclassification of polymorphisms as somatic variants. Clinical data were updated at the time of the second interim analysis (January, 2017).

**Results:** Out of the 300 enrolled patients, 174 were evaluable for mutations. Median follow-up of the cohort was 36 months, and 3-years PFS and OS were 67% and 86%, respectively. Patients not included in the study, due to unavailable tumor DNA (n=126) showed superimposable clinical features and outcome. Mutations of TP53 (8% of cases) and KMT2D (11% of cases) associated with an increase in the hazard of progression both in univariate analysis as well as after adjusting for MIPI, Ki67 and blastoid variant: HR 3.87 (95% CI 1.64 to 9.13), p<0.002 and HR 3.66 (95% CI 1.77 to 7.56), p=0.001, respectively. These results translated into an increase of the hazard of death in both TP53 and KMT2D mutated patients both in univariate analysis as well as adjusting for MIPI, Ki67 and blastoid variant HR 4.26 (95% CI 1.34 to 13.57), p=0.014 and HR 3.09 (95% CI 1.77 to 5.41), p<0.001, respectively.

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

**Figure 1.**

**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.
Multifaceted aspects of bleeding disorders

P305
A LOOKBACK AT VWD TYPE 2A AND 2M CLASSIFICATION IN A LARGE COMPREHENSIVE HAEMOPHILIA CENTRE.
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Background: Von Willebrand Disorder (VWD) has a prevalence of approximately 1% in the general population and is due to quantitative deficiencies or qualitative defects of the Von Willebrand Factor (VWF) protein. VWF is a large multimeric protein with multiple functions. It carries and protects factor VIII and helps in the binding of FVIII, platelets and the vascular endothelium at sites of injury. VWF binding to platelets is through several receptors 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 (VWF:Ag assay) and the function of the protein i.e its ability to bind to 1) FVIII - VWF binding assays), 2) platelets (VWF:Rc0f assay) and 3) collagen (VWF:CB assay). Other tests include ristocetin induced platelet aggregation (RIPA), multimer analysis, assay ratios and VWF genetic analysis. No single commercially available laboratory method can achieve to test all the parameters required to clinch the accurate diagnosis of the subtypes of VWD. Use of those multiple assays with VWF: Rcof/VWF:Ag ratio, VWF CB (VWF-CB) (VWF Ag) ratio have helped in the better identification of VWD and the subtypes.

Aims: To assess recent various VWF investigation panels and assay ratios, VWF genetic analysis, multimeric patterns of the VWF protein in accurate diagnosis of the VWD subtypes. VWD 2A and 2M shows similarities in certain aspects and it is important to differentiate these 2 subtypes as new therapies become available and personalised treatment approaches of VWD become a reality.

Methods: Clinicians who have made a diagnosis of VWD for individuals referred for a bleeding state work up would classify the subtypes of the VWD according to the results of the investigations available at the time of seeing the patients. All patients with an inherited bleeding disorder would then be registered in the centre and details would be put into a database. We have looked back into the database from the period of 2000 to end of 2016 and focussed on the VWD types 2A and 2M. Current VWD diagnostic panel in our centre includes the following tests: FVIII one stage assay, VWF:Ag Elisa,VWF rcof, Platelet agglutination method, VWF CB Elisa methods, VWF multimer analysis by gel chromatography and VWF exon 27/28 genetic mutations are routinely done. New information and new set of results for the registered patients have been taken into account the classification of VWD type 2A and 2M and the database are updated.

Results: In the VWD database 36 patients classified as 2M and 19 patients as type2A have been recorded from 2000 to end of 2016. With the updated results and genetic analysis and the response to DDAVP, around 30% of the patients have had their subtypes changed. This exercise confirms that no singular test had been able to categorise the patients accurately and that 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.

Summary/Conclusions: Pts from this case series experienced few hemorrhagic/thrombotic events. The majority was asymptomatic and the most severe events were related to concomitant pathologies. Nonetheless, this study has the potential to collect data from a numerous population of pts who live in the same country, and therefore to provide useful information to better characterize and manage these rare diseases.

P307
OSTEOPOROSIS IN PATIENTS WITH HEMOPHILIA
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Background: Osteoporosis is often a co-morbidity of hemophilia, which exacerbates the hemophilic arthropathy and affects the long-term stability of the components after the arthroplasty. We present our results for the presence of osteoporosis in 148 patients with haemophilia and hemarthropic arthropathy.

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

Methods: In the period from 2015 to 2016, the presence of osteoporosis surveyed 148 patients with haemophilia who are hospitalized in the department of reconstructive orthopedics for patients with hemophilia (Moscow, Russia): 121 (81.8%) - hemophilia A, 21 (14.2%) - and hemophilia B 6 (4%) – haemophilia patients with inhibitor. The average age of the patients was 39.3 years. (range 10 to 69 years). 121 patients with hemophilic arthropathy performed primary total arthroplasty (98 knee, 20 hip, 3 shoulder joints); 18 patients underwent revision arthroplasty (5 - purulent infection, 7 - instability of the implants, 4 - fractures, 2 - loss of motion in the operated joint). 40 patients underwent ultrasound densitometry. In this series, 13 patients with osteoporosis were identified. As a result of ultrasound densitometry in 17.5% (7 patients) of cases revealed osteopenia and 20% (8) T-thickest index. 105 patients underwent histological study in which 93 (88.6%) bone resorption, 58 (55.2%) cases, bone resorption in patients with hemophilia considerably more common than in the general population. Intraosseous hemorrhage identified in more than half of the cases, exacerbate the decline in bone mineral density.

Summary/Conclusions: The data indicate that osteoporosis at patients with haemophilia considerably more common than in the general population. Intraosseous hemorrhage identified in more than half of the cases, exacerbate the decline in bone mineral density.
patient, with the rate of recurrent haemorrhagic events in joint(s) from 6 to 13 per year. The control group consisted of 255 age-matched healthy men. Gene polymorphism of apolipoprotein E (ApoE e2/e3/e4), paraoxonase (PON1 Gln192Arg), methyltetrahydrofolate reductase (MTHFR C677T), catalase (CAT C-262T) and plasmatic glutathione peroxidase (GPX3 T-65C) 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 -65T/C 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%, 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.

Methods: Library preparation was performed with TruSeq 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, Polymorphism all MutationTaster. Candidate variants were confirmed by Sanger sequencing and family study. For VWF gene, multiplex ligation dependent probe amplification assay was also done using SALSALPRA melanin polynucleotide P011-B3/P012-B3. Among variants from genes of primary interest, common variant with minor allele frequency ≥1% in 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), Bernard-Soulier syndrome (GP1BA), Glanzmann thrombasthenia (ITGAM, ITGAI), Hermansky-Pudlak syndrome (HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC153), Chediak-Higashi syndrome (LYST), Griscelli syndrome (MYO5A, RAB27A, MLPH), Scott syndrome (ABC4A).

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 (c.1040_1046delinsAla) in ITGB3. The former was pathogenic which resulted 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.399C>T (p.Pro1127_Gly1180delinsArg) in VWF, especially the latter synonymous variant previously confirmed to be resulted in exon 26 skipping. Another proband had a novel variant, c.2008C>T (p.Arg670Cys). The last proband had a known VWF pathogenic variant of c.1728G>T (p.Met576Ile).

Summary/Conclusions: DES is a valuable method to confirm GT or VWD. Further study is needed to find out unidentifiable mutations by this strategy.
AN ALGORITHM TO IDENTITY CASES OF SEVERE HEMORRHAGE IN ROUTINELY COLLECTED HEALTHCARE DATA

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.

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

Background: Previously we determined common deleted region (CDR) of del(20q) observed in MDS by CGH-array. Our data showed that the PTPN1 gene is located within CDR of del(20q). The PTPN1 gene encodes PTP-1B, a non-receptor type protein tyrosine phosphatase, which is involved in multiple physiological and pathological cellular processes via dephosphorylation of several tyrosine kinases, and other molecules. Although roles of PTP-1B in normal and pathological hematopoiesis has not been elucidated, it may function negative regulator for cellular processes mediated by tyrosine kinases, including Jak2, and SRC. We hypothesized that the PTPN1 gene is a target gene disrupted by del(20q), resulting in haplo-insufficiency, and involved in MDS molecular pathogenesis.

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

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

Results: A total of 118 MDS patients, 71 males and 47 females with median age of 68 years (range: 20-91 years) and 19 control subjects were included in the present study. The patients were classified as RCUD (n=18), RCRM (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=0.2021). Median values of relative PTPN1 expression level in RCUD, RCRM, 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, RCRM, RARS) (median value: 1.41 vs 1.89, P=0.0074). To investigate prognostic implication of PTPN1 expression in MDS, we analyzed impact of PTPN1 expression on overall survival (OS). Based on PTPN1 expression level, 118 patients were divided into four groups, high (Q1), intermediate (Q2, Q3), and low (Q4) quartiles. Kaplan-Meier analysis demonstrated that the lowest quartile (Q4) showed significantly worse survival compared with remaining quartiles (Q1, Q2, Q3) (P=0.048). The estimated 5-year OS rates in Q1-3 group and Q4 group were 69% and 49.8%, respectively. We examined whether PTPN1 expression is induced by 5-azacytidine in primary bone marrow cells of 17 MDS patients. Real-time PCR analyses indicated that 5-azacytidine treatment significantly induced PTPN1 expression.

Summary/Conclusions: The present study demonstrated that PTPN1 expression is reduced in MDS patients with haplo-insufficiency due to del(20q) and methylation of promoter region of the PTPN1 gene. Low PTPN1 expression is associated with advanced disease and poorer clinical outcome, indicating that PTPN1 expression level could be a useful prognostic marker in MDS.

MOLECULAR MARKERS PREDICTING RESPONSE TO AZACITIDINE TREATMENT FOR MYELODYSPLASTIC SYNDROMES

Background: MDS is a clonal hematopoietic malignancy characterized by ineffective hematopoiesis and varying degrees of dysplasia leading to cytopenia and/or excess blasts (EB). Proliferation of pathologic hematopoietic cells is driven by genetic and epigenetic factors. Among these, activation of the JAK-STAT signaling pathway has been implicated in the pathogenesis of MDS. JAK2, a non-receptor tyrosine kinase, is mutated in ~20% of patients with MDS. Therefore, it is important to identify JAK2 mutation status as a biomarker for the prediction of treatment response to targeted therapy. Azacitidine is a demethylating agent that induces hypomethylation of DNA and re-expression of silenced genes. However, the role of epigenetic changes in the prediction of treatment response to azacitidine remains unclear.

Aims: The aim of this study was to investigate whether epigenetic changes, particularly DNA methylation, in concert with gene expression changes, can predict the response to azacitidine treatment in patients with MDS.

Methods: A total of 40 MDS patients were enrolled in this study. DNA methylation was assessed by bisulfite genomic sequencing in 300 genes, including JAK2, and gene expression was measured by RNA sequencing. The response to azacitidine treatment was defined as complete remission or partial remission according to the International Working Group criteria. Univariate and multivariate analyses were performed to identify potential biomarkers of response to azacitidine treatment.

Results: DNA methylation levels in the promoter region of JAK2 were significantly lower in responders compared to non-responders. Moreover, high expression of the epigenetic modifier DNMT3B and low expression of the tumor suppressor genes TP53 and PTEN were associated with treatment resistance. Multivariate analysis identified JAK2 methylation and TP53 expression as independent predictors of response to azacitidine treatment.

Summary/Conclusions: Our findings suggest that epigenetic changes, particularly DNA methylation and gene expression, can predict the response to azacitidine treatment in patients with MDS. These biomarkers may help to identify patients who are likely to benefit from azacitidine therapy, thereby optimizing treatment strategies.
Background: DNA hypomethylating agents (HMAs) comprise standard therapy for non-transplant-candidate high-risk myelodysplastic syndromes (MDS). However, little is known about the exact mechanism of their effects to MDS or their clinical utility and response to azacitidine (AZA).

Aims: The purpose of this study is to elucidate the clonal dynamics and molecular signatures that correlate with response to azacitidine therapy for MDS, focusing on the role of TP53-mutations.

Methods: We conducted a prospective multicenter trial of azacitidine treatment for high-risk MDS patients in which the efficacy was compared between the 5-day and 7-day regimens. A total of 107 patients were enrolled between 2013 and 2016. For all cases, a bone marrow specimens collected before therapy was analyzed for mutations using targeted-capture sequencing. Mutations were also interrogated after 4 cycles of azacitidine therapy in 48 (45%) cases. An additional 24 cases was also analyzed for mutations who received azacitidine therapy for MDS and whose bone marrow specimens were available both before and after therapy.

Results: On average, 2.7 mutations (range 0-9) were detected per sample before azacitidine treatment. TP53 represented the most common mutational target (29% cases) 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) (5.6%) and 19 marrow CR (17.8%) and 7 (29%) cases (all CR) in the off-protocol cohort. Notably, CR was obtained almost exclusively in TP53-mutated cases (5/6 and 5/7 CR cases in the on- and off-protocol cohort. No other mutations were associated with clinical remission. Median duration to CR was 119 days (range 81-721), which lasted for a median duration of 217 days (range 10-783). DeltaT (tumor cell fraction) was evaluable for 62 cases who had one or more low-up samples and carried at least one mutation in either pre- or post-treatment. Among with an average of 0.075 (range -0.75-0.72). DeltaT was significantly lower in responders than non-responders (-0.18 vs -0.0002, p=0.0068) and in TP53-mutated cases (-0.25 vs 0.0008, p=0.001).

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 treatment response seems to be an open question for this clinical trial. The study results suggest a potential role of TP53 as a key mediator to regulate the clonally selected clonal dynamics of TP53-mutated tumors. Further studies should be warranted to confirm its efficacy and to develop an optimal post-remission therapy to overcome the short remission period.

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

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

Methods: We conducted a single arm phase II clinical trial of guadecitabine at a dose of 60mg/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. Response included hemoglobin response for anemia, improvement for lineages for neutropenia and thrombocytopenia.

Results: A total of 53 patients have been enrolled: 50 (94%) are evaluable for toxicity and 44 (83%) for response. Median age is 67 years (49-87). A total of 43 (86%) patients have MDS and 7 (14%) have CMML. A total of 21 (42%) patients had a history of therapy for other hematologic malignancies (HMA). The most frequent type of mutation in patients with TP53 mutations being the most frequently detected in 36% patients. After a median of 6 treatment cycles (1-20), the ORR is 71% including 32% CR. Median response occurred by 3 cycles (1-6). Seven (21%) out of 33 evaluable patients achieved a complete cytogenetic response. Ten (20%) subjects proceed to allogeneic stem cell transplantation. Median follow-up was 3.3 months (0-23). Median OS is 14.1 months (CI 13.3-14.9 months) and median EFS is 8.4 months (CI 5.6-11.2 months). Forty-five (90%) patients experienced at least one AE during therapy. Most common grade 1-2 AEs included fatigue (66%), nausea (38%) and dyspnea (26%). Dose reductions due to cytopenias were required in 17 (34%) patients. Early 8-week mortality occurred in 3 (6%) patients.

Summary/Conclusions: Guadecitabine is well-tolerated and active in patients with higher-risk MDS and CMML even in the presence of adverse biological features such as high frequency of complex karyotype, treatment related disease and TP53 mutations.
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with non complex del(7q) (P=ns for complex vs non complex, chi-square test).
The ORR was 37.5% in “de novo” and 38.4% in secondary MDS, respectively
(P=ns). Impact of AZA treatment compared to BSC on overall survival: Results
of this multivariable analysis of OS at different time points are presented in
Table 2. 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).

serum were >200 h at 5 mg/kg and >300 h at 10 mg/kg. There was a greaterthan-dose proportional increase in mean OPN-305 exposure (AUC) between
5 and 10 mg/kg. PK profiles after repeated dosing at 5 mg/kg in N=2 subjects
and pre-dose (trough) levels in other subjects indicated some variability in the
potential for accumulation. TLR-2 receptor occupancy in blood PBMCs and
bone marrow aspirates was complete in virtually all samples taken after OPN305 administration. There is no evidence of treatment related anti-drug antibodies. Compared with baseline, no significant changes of IL-23, IL-18, IFN-r,
IL-10, IL-1β, 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,
with no differences in response based on cytogenetic or mutational profile.
Summary/Conclusions: Treatment with OPN-305 in pts with previously treated lower-risk MDS was well tolerated with no significant toxicities and 53%
ORR including 20% transfusion independence, and potential association
between TLR2 levels and response.
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Figure 1.

Summary/Conclusions: This study confirms the benefit of AZA treatment on
outcome in patients with HR-MDS and cytogenetic abnormalities involving
chromosome 7.
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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
MYELODYSPLASTIC SYNDROMES WITH PRIOR HYPOMETHYLATING
AGENT THERAPY
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Background: Alterations of innate immune signaling, including overexpression
of TLR2, are common in MDS. Significant TLR2 overexpression in MDS bone
marrow CD34+ cells, especially after HMA therapy, has been reported. OPN305 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: To evaluate the potential therapeutic value of OPN-305 in patients (pts)
with MDS
Methods: We designed a phase I/II trial of OPN-305 for pts with Low or Int-1
risk MDS by IPSS after failure to prior therapy with a HMA (≥4 cycles). Pts
where required to be transfusion dependent (≥2 units in 8 weeks). Pts with isolated del(5q) should have received therapy with lenalidomide. Because, OPN305 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.
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 normal karyotype, 2 del(5q), 2 trisomy 8, 1 del(20q), 1 monosomy Y and
2 other single or double abnormalities. Median number of prior therapies was
2 (range 1-4) with a median duration of prior therapies of 23 months (range 656). A total of 5 (29%) pts developed AEs related to OPN-305. All AEs were
grade 1 with gastrointestinal disorders being the most frequent (23%). At this
point, no significant drug related toxicity has been documented with no excess
infectious complications. Overall response rate in the form of hematological
improvement was 53% (8/21) with 3 (20%) pts achieving transfusion independence and 5 (33%) minor hematological improvement. Half-lives of OPN-305 in

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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
prognosis.
Methods: The results of HSCT in 115 MDS patients from five centres in Spain
were retrospectively analyzed. Bone marrow samples were collected a median
of 27 days prior to transplant and DNA was screened for somatic mutations by
NGS, using a NextSeq platform (Illumina). Two myeloid genes panels that
included the most frequently mutated genes in myeloid malignancies were
used.
Results: Median age was 53 years (range from 19 to 70). Fifty-eight percent
were male and 79.13% were classified as de novo MDS. According to WHO
2008 classification 4 (3.5%) were RCUD, 2 (1.8%) RARS, 22 (19.50%) RCMD,
28 (24.8%) RAEB-1, 32 (28.3%) RAEB-2, 12 (10.6%) Unclassifiable MDS, 9
(8%) CMML 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
(18.8%) had very high risk; 9 patients with CMML (10.6%) were categorized
separately. Among patients with known karyotype (101 of 115), 7 of them (6.9%)
had a complex kariotype (CK). Regarding mutational study, 44 patients (38.3%)
didn’t shown any mutation before transplant; 27 patients (23.5%) had 1 mutated
gene, 15 (13%) had 2, 19 (16.5%) had 3, 6 (5.2%) had 4, 3 (32.6%) 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%), TET2 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).
Patients were divided into 2 groups: group 1, with 2 or less mutated genes
(74.5%) and group 2, with more than 2 mutated genes (25.2%). Group 2 had
a lower OS (46.9% vs 69.6% at 1 year; p=0.035) and a higher Cumulative Incidence of Relapse (CIR) (25.3% vs 10.1% at 1 year; p=0.007). Development of
cGVHD significantly improved outcome in both groups (Figure 1). Univariate
analysis determined that developing of cGVHD, CK, number of mutated genes
(more than 2 mutated genes) and mutations in TET2 significantly impacted on
outcome. Nevertheless, only the development of cGVHD as a time-dependent
variable (HR 0.046, 95%CI 0.016-0.138, p<0.001) and TET2 mutations (HR
2.562, 95%CI 1.018-6.447, p=0.046) significantly influenced on OS in multi-


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).

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 responses rates comparable or better than those generally observed with azacitidine alone. Additionally, the transplant rate observed was encouraging in this patient population.
Myeloma and other monoclonal gammapathies - Biology

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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 M3P (v2.0 or v3.0) gene selection, that includes most currently known functional Ps targets as well as the most common known IMiD targets. Functional significance was validated using different functional assays.

Results: Our analysis included five genes each with known association to drug response to IMiDs (CRBN, C4BL4, IKZF1, IKZF3 and IRF4) and Ps (PSMB5, PSMB8, PSMB9, PSMD1 and XBPF1). Based on the increased sequencing depth, the mutation incidence in untreated patients is higher than in the CoMMPass dataset (IMiDs: 5.8% vs 3.9%; Ps: 1.9% vs 1.4%). Furthermore, pretreated patients showed a significant mutational increase compared with untreated pts (IMiDs: 19.7%, Z-score: -4.2, p<0.001; Ps: 7.3%, Z-score: -2.6, p=0.009). We observed a Gly159Arg mutation within the Lenalidomide (Len) degron sequence of IKZF3 in a patient progressing on Len and Pomalidomide (Rom), as well as two XBPF1 truncating mutations in PI refractory patients. Of note, among three treated cases with mutations in the 85 (PSMB5) or 85 (PSMB8) PI binding subunit of the proteasome, one patient harbored not less than 4 subclonal mutations. This is the first description of PSMB5 mutations in human MM, identified in a patient with long term history of PI treatment. All mutations were located in or close to the Bor binding site of PSMB5. The functional analysis demonstrated induction of resistance not only to Bor (IC50/PSMB5wt= 2 nM vs IC50/PSMB5mut= 4.5-8 nM), but also to the second generation PI Ixazomib (IC50/PSMB5wt= 5.2 nM vs IC50/PSMB5mut= N/A) and Carfilzomib (IC50/PSMB5wt= 8 nM vs IC50/PSMB5mut= 13-22 nM). Of interest, the P97 blockade of the protein homeostasis by the investigational compound CB5083 remains still possible in the mutated cell lines and the resistance can be overcome. Finally, Pom treatment eradicated two of the PSMB5 containing subclones (Figure 1).

Summary/Conclusions: Under the selective pressure of anti-MM therapy the incidence of mutations in genes associated with drug resistance increases in previously treated MM patients. 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 were enlisted into an in vitro screening strategy that employed a single-stranded DNA-PCR-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, INT3S, LASS2, KRTCAP2 and ILF2 as potential 1q21-specific vulnerability targets whose expression is driven by copy number functionally validated, both in vitro and in vivo, Interleukin-2-enhancer binding factor 2 (ILF2) as a key 1q21 amplification-specific gene. Our results show that ILF2 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
effectors, which enables genomic instability, promotes adaptive mechanisms to genotoxic stress, and enhances cell survival, thereby promoting drug resistance and disease progression. Given that 1q21 amplification is one of the most frequent copy number alterations in cancer, synthetic lethality approaches based on targeting gain-of-function associated to ILF2 may have a broad spectrum of application to potentiate the sensitivity of cancer cells to chemotherapeutic agents.

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

**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), United States, Italian Multiple Myeloma Network, GIMEMA, Unit of Clinical Epidemiology, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino and CPO Piemonte, Torino, Italy, 2Multiple Myeloma Research Foundation, (MMRF), United States, 3Italian Multiple Myeloma Network, GIMEMA, 4Unit of Clinical Epidemiology, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino and CPO Piemonte, Torino, Italy. 5Myeloma Unit, Division of Hematology, University of Torino - Currently Takeda Pharmaceuticals Co., Torino, Zurigo, Italy, Switzerland

**Aims:** We hypothesize that specific genes present in the 1q minimal amplified region are critical regulators of clonal evolution and tumor progression in MM. Gain of 1q is one of the most frequent copy number alterations in cancer, synthetic lethality approaches based on targeting gain-of-function associated to ILF2 may have a broad spectrum of application to potentiate the sensitivity of cancer cells to chemotherapeutic agents.

**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 transplant. Results: 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 versus I. The prognostic trend of the score was confirmed in different patient subgroups including ASCT/no ASCT, standard/high risk cytogenetic profile, ISS I, II, or III. Of note, 23% of patients in group I had ISS III and 34% of patients in group IV had ISS I.

**Table 1.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Mutated Yes/No</th>
<th>Score assigned</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRR2C</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>UH2A</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>RBP3</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>PKHD1</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>HNRRN</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>FAT4</td>
<td>No</td>
<td>2</td>
</tr>
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<td>KRA5</td>
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<td>3</td>
</tr>
<tr>
<td>NRAS</td>
<td>Yes</td>
<td>3</td>
</tr>
</tbody>
</table>

**Figure 2.**

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

**P323**

TARGETING GENE DEPENDENCY OF 1Q AMPLIFICATION IN MULTIPLE MYELOMA

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**Background:** Gain of 1q is one of the most frequent copy number variations across cancer types and in Multiple Myeloma (MM). Gain of 1q is associated with a poor outcome, indicating it is a potential driver in MM progression and resistance to treatment. While the whole 1q arm can be amplified in some cases, a specific minimal amplified region has been identified by CGH array, including approximately 500 genes in the 1q21.1-23.3 region. However, the driver genes in the 1q region are unknown.

**Aims:** We hypothesize that specific genes present in the 1q minimal amplified region are critical regulators of clonal evolution and tumor progression in MM.

**Methods:** To explore gene dependency in 1q21.1-23.3 in MM, lung, breast and
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DUAL INHIBITION OF DNM1 AND EZH2 CAN EFFECTIVELY OVERCOME BOTH INTRINSIC AND ACQUIRED RESISTANCE OF MYELOMA CELLS TO IMiDS

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Background: The introduction of novel agents for the treatment of multiple myeloma (MM), mainly proteasome inhibitors and immunomodulatory agents (IMiDs), has significantly improved the survival rates of the patients, and both classes of drugs stand as the main treatment options for MM. Several studies have identified Cereblon (CRBN) as the direct target of not only thalidomide, but also lenalidomide and pomalidomide, and suggested that its expression is essential for the anti-myeloma effect of these drugs. However, even though the expression levels of CRBN have been associated with response to IMiDs, the precise mechanisms behind IMiD resistance still remain elusive.

Aims: The aim of this study was to determine the expression of epigenetic modifications in MYELOMA CELLS TO IMiDS, and identify novel therapeutic targets in MM.

Methods: For the development of IMiD-resistant cell lines (OPM2-LR and -PR, H929-LR and -PR), we treated OPM2 and H929 continuously with increasing doses of either lenalidomide or pomalidomide for 4-6 months, until cell viability decreased in chromatin accessibility and to a much lesser extent with DNA methylation changes. Interestingly, neither CRBN nor any of the other molecules involved in the CRBN pathway (IKZF1, IKZF3, IRF4) i showed increased sensitivity to both lenalidomide and pomalidomide, with significantly impaired proliferation of gain of 1q cells. Several candidate genes were identified as being the top genes preferentially affecting the proliferation of gain of 1q cells. To further confirm our candidate genes were overexpressed in gain of 1q patients, we studied publicly available gene expression profiling from the Multiple Myeloma Genomic portal (MMGP) and the Cancer Genome Atlas (T1). Using list of candidate genes identified a large expression profiling resource developed by the Library of Integrated Network-based Cellular Signatures (LINCS) program to identify potentially active drug targeting our candidate genes. Finally, a targeted drug screening was performed using 179 compounds identified through the LINCS program and using the library of compounds, 17 compounds were found to target Cereblon. These data were used as input for a drug screen that was developed and validated in 1q+ (OPM2, H929 and KMS11) and non 1q 1q cells (KMS18).

Results: We were able to identify 10 candidate genes, for which knockdown significantly impaired proliferation of gain of 1q cells. Five of these genes showed a significant increase in expression in both patient samples and controls, naive B cells, germinal center B cells, memory B cells and plasma cells. Data were extensively mined using a battery of different bioinformatic tools. 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-Aza and 5-Azacytidine. However, in vivo experiments are required to confirm these findings and to make them very good candidates for a prospective phase I study to examine their potential as "IMiD-resensitizers", which may improve the outcome management of MM patients with drug-resistant myeloma clones and a potentially high-risk disease.

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MULTILAYER EPIGENOMIC ANALYSES REVEAL OF NEW CANDIDATE ONCOGENES INVOLVED IN THE PATHOGENESIS OF MULTIPLE MYELOMA

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Background: Most of the published omics studies in multiple myeloma (MM) have focused on the analysis of the genome, transcriptome and DNA methy- lome. Over the last years, the chromatin structure and histone modifications are emerging as essential epigenetic layers to understand gene deregulation in MM. Although this field remains widely unexplored in MM.

Aims: We herein aim to elaborate a comprehensive description of the MM epigenome including multiple layers of information.

Methods: We performed ChIP-seq of six histone modifications with non-overlapping functions (H3K4me3, H3K4me1, H3K27ac, H3K36me3, H3K27me3, and H3K9me3), ATAC-seq for chromatin accessibility, Whole Genome Bisulfite Sequencing (WGBS) for DNA methylation, and RNA-seq for gene transcription in purified bone marrow plasma cells from 4 MM patients and, as healthy controls, naive B cells, germinal center B cells, memory B cells and plasma cells. Data were extensively mined using a battery of different bioinformatic tools. 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-Aza and 5-Azacytidine. However, in vivo experiments are required to confirm these findings and to make them very good candidates for a prospective phase I study to examine their potential as "IMiD-resensitizers", which may improve the outcome management of MM patients with drug-resistant myeloma clones and a potentially high-risk disease.
cells. Out of this list, we observed that two adjacent genes, PRDM5 and NID2, were co-activated in MM. The analysis of their expression in additional patient cohorts indicated that their co-activation is a consistent event in MM pathogenesis and that their levels were negligible in bone marrow and tonsillar plasma cells. When analyzing chromatin topology by 4C-Seq, we identified 3D interactions between both gene loci only in MM cells, suggesting that DNA looping between the two genes may be related to their co-activation in MM. Finally, knockdown of each of these genes using inducible shRNAs, decreased cell proliferation and induced apoptosis in MM cells.

Summary/Conclusions: Collectively, our initial exploration of histone modification profiles in MM has revealed an extensive activation of the MM chromatin landscape, which harbors many candidate oncogenes. Reversing this global activation by epigenetic drugs, such as BET inhibitors, may represent an attractive therapeutic option for MM.

Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>VAF (%)</th>
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<tbody>
<tr>
<td>IDH1</td>
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<tr>
<td>IDH2</td>
<td>34.5</td>
</tr>
<tr>
<td>ETF2</td>
<td>5.0</td>
</tr>
<tr>
<td>ETF6</td>
<td>2.3</td>
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<tr>
<td>CBL</td>
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</table>

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CLINICAL IMPLICATIONS OF CD34+ CELLS, STEM CELL HARVEST FROM PATIENTS WITH PLASMA CELL DYSCRASIAS

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Background: Introduction of novel treatments; Lenalidomide, high-dose alkylating agents (Melphalan) conditioning prior to autologous stem cell transplant (ASCT) over the last few decades has improved overall survival in patients with Multiple Myeloma (MM). In spite of enhanced survival rates, some hematological malignancies (SPM) like Myelodysplastic Syndrome (MDS) and Acute myeloid Leukaemia (AML). Clonal haematopoiesis resulting in sequential accumulation of genomic events and their combination with a driver-passenger genetic mutations (in upto 80% of MDS & >95% AML patients) steer MDS/AML disease pathogenesis and clinical outcome. Therefore, we hypothesised that detection of Clonal Hematopoiesis of Indeterminate Potential (CHIP) in haematopoetic stem cells (HSCs) prior to ASCT in patients with MM treated with a range of therapies could be utilised for predicting patients at risk of developing SPMs i.e. MDS/AML.

Aims: To ascertain baseline mutational spectrum [especially low-level clones with variant allele frequency (VAF) ≥5%] of MDS/AML associated gene mutations in HSCs prior to ASCT in order to predict patients at risk of clonal evolution, transformation to MDS/AML.

Methods: DNA was isolated from mononuclear cells (MCs) collected by leukopheresis prior to ASCT from 128 MM patients. A customised amplicon-based Illumina MiSeq panel was used for the sensitive interrogation of 24 most common genes harbouring mutations in MDS/AML (splicing factor genes; SRSF2, ZRSR2, genes implicated in epigenetic regulation; TET2, IDH1, IDH2, ASXL1, EZH2 & DNMT3A, known non-coding genes involved in cell signalling/transcription regulation and cohesion complex; TP53, FLT3, NRAS, KRAS, ETF6, RUNX1, CCBl, CKIT, JAK2, MPL, CEBPA, STAG2, GATA2, KDM6A and NPM1). Variant analysis was performed using Illumina Variant Studio (≥5% VAF & read depth ≥150X thresholds for Myelodysplastic Syndromes (MDS) and 5% VAF for Acute Leukaemia (AML)). VAF range 7-50% in DNMT3A, IDH1, IDH2, TET2, ETF6 and CBL genes (Table 1). Four missense mutations identified in DNMT3A were aggregated in the Mtas e domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function. Previous studies confirm R882 variant in the Mtase domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function. Previous studies confirm R882 variant in the Mtase domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function.

Results: Seven patients (6.25%) contained heterozygous somatic mutations (VAF range 7-50%) in DNMT3A, IDH1, IDH2, TET2, ETF6 and CBL genes (Table 1). Four missense mutations identified in DNMT3A were aggregated in the Mtas e domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function. Previous studies confirm R882 variant in the Mtase domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function. Previous studies confirm R882 variant in the Mtase domain responsible for its methyltransferase activity indicating a strong intent to abrogate this function.

Summary/Conclusions: Our data identifies for the first time a subgroup of MM patients (6.25%) with no morphological evidence of MDS/AML prior to ASCT but harbouring CHIP in CD34+ harvest stem cells and later developing MDS/AML. These findings are pivotal for identification of such patients at risk of clonal evolution and transformation prior to ASCT since it can be a significant parameter in determining appropriate treatment modality i.e. whether or not to employ CHIP harbouring CD34+ harvest stem cells as therapy for these patients.

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PATHOPHYSIOLOGICAL FUNCTIONS AND CLINICAL IMPACT OF THE NEW IMMUNORECEPTOR SLAMF3 IN MULTIPLE MYELOMA

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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/refractory 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 of MM cells to antimalyeloma 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 (sASM) 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 antimalyeloma agent-induced apoptosis in SLAMF3hi MM cells were significantly higher and lower than in SLAMF3low cells, respectively. The cell proliferation and drug resistance in SLAMF3-expressing KMS34 cells were promoted in comparison with SLAMF3low cells. That malignant potential in MM cells was cancelled by SLAMF3 knockdown. Furthermore, the proliferation of MM cells and resistance to antimalyeloma agents were inhibited by anti-SLAMF3 antibody. Adaptor proteins, SHP2 and GRB2, were expressed in MM cell lines, but neither SAP nor EAR-2 were. SLAMF3 interacted directly with SHP2 and GRB2, and SHP2 also interacted with GRB2. SHP2 inhibitor-treated or SHP2/GRB2-knockdown cells had characteristics similar to SLAMF3-knockdown cells.

3) The frequency of GG genotypes of SLAMF3 SNP rs509749 in MM patients was 63.6% (n=28), of AG 29.5% (n=13), and of AA 6.8% (n=3). Patients with GG genotypes tended to have shorter overall survival times compared to patients with AG genotypes.

4) sSLAMF3 levels were significantly higher in symptomatic MM than in asymptomatic MM and markedly increased in advanced MM. MM patients with high levels (≥3.3 ng/mL; n=62) of sSLAMF3 progressed to the
advanced stage significantly more often and had shorter progression-free survival times than those with low-levels (p=0.0012).

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 human antibody (SP7219) conjugated covalently to the non-natural amino acid para-azido-methyl-L-phenylalanine (pAMF) with a non-cleavable dibenzo cyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by novel cell-free antibody production and click chemistry results in a well-defined homogeneous ADC drug product with a drug-antibody ratio (DAR) of 2.

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

Methods: DBCO-Alexa647-conjugated SP7219 staining and flow cytometry were used for detection and quantitation of CD74 expression on MM cell lines. STRO-001 was used to determine the EC50 and percent span of killing in MM cell lines. The anti-tumor activity of STRO-001 was evaluated in the disseminated ARPE-1 and MM.1S MM models. In vivo bioluminescence imaging (BLI) for animals bearing MM.1S-luc cells was performed using an IVIS Spectrum. BLI images were collected 7, 14, 21, and 28 days post-tumor inoculation. STRO-001 was administered to cynomolgus monkeys in an exploratory dose-escalating study of repeat IV doses of 1, 3, 10 and 30 mg/kg on days 1 and 15.

Results: In vitro cytotoxicity assays show nanomolar potency of STRO-001 in five MM cell lines: MC/CAR (EC50 0.8 nM), ARD (EC50 6.5 nM), MM.1S (EC50 10-11 nM), U266B1 (EC50 8.5-9.3 nM), and ARP-1 (EC50 4.3-22 nM). CD74 cell surface expression is required for STRO-001 cytotoxic activity but expression level, as measured by antibody-binding capacity, does not correlate strongly with in vitro potency (R²=0.5837 for MM cell lines). STRO-001 inhibits the growth of various MM cell lines. STRO-001 at 10 mg/kg on day 1 and 15 showed 100% tumor burden reduction in the MM.1S disseminated model. At termination of the study, 129 days post-inoculation, 100% of the STRO-001 treated animals survived and showed no evidence of disease with no CD138+ cells in their bone marrow, while mean survival of vehicle-treated control animals was 35 days with 50% of their bone marrow containing myeloma cells. BLI of luciferase-expressing MM.1S (MM.1S-luc) tumor cell lines enabled non-invasive quantitation of tumor burden. Single doses of 1.3, and 10 mg/kg STRO-001 (administered on day 7 post-inoculation) resulted in eradication of myeloma by day 28 based on bioluminescence signal and quantification of CD138+ cells in bone marrow. In addition, STRO-001 produced a dose-dependent reduction in normal B-cells in cynomolgus monkeys, providing pharmacodynamic evidence of B-cell targeting (Figure 1).

Summary/Conclusions: STRO-001 demonstrates potent in vitro cytotoxicity in MM cell lines and reduces tumor burden in MM xenograft models, including significant prolongation of survival in the MM.1S model. Based on these encouraging observations, STRO-001 is advancing to the clinic for the treatment of CD74-expressing B-cell malignancies.

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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 95.6% and a Phred quality score of 91.3% up to Q30. Data were analyzed with a custom pipeline and 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, HNRP-NPL), adherent junction function (RASSF8), GTPases (RAPGEF2, RAB40A), and genes of the collagenase family (COL9A1, COL1A2) among others.

Summary/Conclusions: Taken together, these results suggest that the mutation pattern in AL is heterogeneous with no common mutated gene among all patients. However, we described novel mutations in the context of AL in regulatory genes or over-representing cancer-related pathways that can help to elucidate the molecular biology of the disease.

Figure 1.
Myeloma and other monoclonal 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-year survival for 4 calendar periods: 1973-1982, 1983-1992, 1993-2002, and 2003-2013 and furthermore for 6 age categories at diagnosis (0-40, 41-50, 51-60, 61-70, 71-80 and >80). Short-term survival, as defined by RSR of less than 3 months, was also defined for all calendar periods.

Results: A total of 21,465 patients (54% males, median age at diagnosis 72 years) with MM were recorded in the time period. Overall, the 1- and 5- and 10-year RSRs improved in the whole period, with the greatest improvement in the two most recent calendar periods. The 1-year RSR increased significantly between all calendar periods (0.69, 0.74, 0.77 and 0.82, respectively). The 5-year RSR increased significantly between the two last calendar periods (0.28, 0.31, 0.33 and 0.41, respectively; Figure 1) as well as the 10-year RSR (0.10, 0.12, 0.14 and 0.20, respectively). Short-term survival increased significantly between the first two and last two calendar periods (the RSR were 0.83, 0.88, 0.89 and 0.93 respectively). Females had a lower excess mortality compared to males (excess mortality ratio 0.91).

Figure 1.

Summary/Conclusions: In this population-based study, based on more than 21,000 MM patients diagnosed during more than a 40-year period, we showed that with an increased use of novel agents in MM patients, survival has improved significantly. This is especially prominent during the last 10 years. Our findings are important, since new agents are approved based on clinical trials, where certain groups, such as older patients and patients with significant comorbidities are often excluded.

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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 del(17p), t(4;14), t(14;16), t(14;20), del(17p), and gain(1q). Bone marrow aspirates were evaluated for deletions, monosomies, trisomies, and tetracisomies using chromosome- or centromere-specific FISH probes. IGH rearrangements were evaluated using an IGH break-apart probe and evaluating up to 5 potential partners (FOFR3, CCND1, CCND3, MAF, and MAFA). 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), 70% (60% of the patients were male. There were 372 HRA in 327 patients (28% of the cohort): 170 (45%) del(17p), 110 (29%) t(4;14), 45 (12%) t(14;16), 8 (2%) t(14;20), and 12 (2%) gain(1q). Of the 280 patients with 1 HRA 130 (46%) had del(17p), 120 (43%) had a high-risk translocation, and 30 (11%) had gain(1q). Of the 46 patients with 2 HRA 34 (76%) had del(17p) and a high-risk translocation, 6 (13%) had a high-risk translocation and gain(1q), 5 (11%) had del(17p) and gain(1q), and 1 had 2 high-risk translocations. There was 1 patient with 3 HRA: del(17p) and t(4;14) and gain(1q). The median overall survival was 6.6 years (6.0 - 8.0) for the entire cohort (n=1181), 8.3 years (6.7 - 8.9) for those without HRA (n=854, 72%), 4.8 years (3.9 - 5.6) for those with one HRA (n=280, 24%), and 2.7 years (2.1 - 3.8) for those with 2 or more (2+) HRA (n=47, 4%). Figure 1 shows the Kaplan-Meier overall survival estimates stratified by the number of HRA (n=1181). The presence of 1 HRA (versus 0, HR 1.57, 95% CI 1.26 - 1.96, p <0.001, n=1181) and the presence of 2+ HRA (versus 1, HR 3.37, 95% CI 2.21 - 5.14, p <0.001, n=1181) were of prognostic significance after adjusting for age, sex, ISS stage, and first-line therapy. When adjusting for the revised ISS instead of the ISS the hazard was attenuated for 1 HRA (versus 0, HR 1.42, 95% CI 1.12 - 1.80, p=0.004, n=1087) and 2+ HRA (versus 1, HR 2.82, 95% CI 1.81 - 4.40, p <0.001, n=1087).

Figure 1.

Summary/Conclusions: Approximately 1 in 4 patients with newly diagnosed multiple myeloma presented with 1 HRA at the time of diagnosis, approximately 1 in 25 with 2 or more HRA. These patients experienced inferior overall survival suggesting a cumulative effect of multiple HRA.
LENLADOMIDE 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 2016) 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, 0.25-1.13). Sensitivity analyses showed consistent results. Updated data will be presented at the meeting.

Summary/Conclusions: Adjusting for the potential diluting effects of crossover reduced median OS and PFS with PBO, and improved the treatment effect in the ITT analyses for OS and PFS for LEN vs PBO maintenance after SCT. The statistical significance of the ITT analyses was maintained throughout.

P334 Efficacy and Safety of Daratumumab, Lenalidomide, and Dexamethasone Versus Placebo Alone in Relapsed or Refractory Multiple Myeloma: Updated Analysis of POLLUX

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Background: Daratumumab, a human monoclonal antibody targeting CD38, significantly prolongs progression-free survival (PFS) and achieves deep and durable responses when combined with other established standard-of-care regimens. In the randomised, phase 3, randoomised study of DRd versus Rd in RRMM.

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

Methods: Eligible patients with ≥1 prior line of therapy were randomly assigned to Rd (25 mg PO lenalidomide on Days 1-21 of each 4-week [Q4W] cycle) or DRd (25 mg PO dexamethasone on days 1, 8, 15 and 22 of each 28-day cycle). In both arms, patients received lenalidomide 25 mg PO on days 1, 8, 15, and 22 of every 28-day cycle. RD was assigned on day 1 of each cycle. Patients crossed over to DRd if they discontinued Rd because of progressive disease, grade ≥3 adverse events (AEs) or at investigator discretion. Grade ≤3 AEs were recorded. PFS was the primary endpoint. Safety, tolerability, and efficacy were assessed in all patients who received ≥1 dose of study treatment. Statistical analyses were based on an intent-to-treat (ITT) population.

Results: A total of 361 patients were enrolled and treated at 102 sites in 12 countries. Of these, 211 patients were randomly assigned to the Rd arm and 150 patients to the DRd arm. PFS was superior in the DRd arm compared with the Rd arm (HR, 0.56; 95% CI, 0.41–0.77; P <0.0001). Median PFS in the DRd arm was 22.6 months (95% CI, 20.9–24.0) versus 13.9 months (95% CI, 11.1–15.4) in the Rd arm (HR, 0.56; 95% CI, 0.43–0.74; P <0.0001). Median TTP was 21.6 months (95% CI, 17.6–23.7) in the DRd arm versus 14.6 months (95% CI, 12.8–16.4) in the Rd arm (HR, 0.57; 95% CI, 0.45–0.73; P <0.0001). Overall response rates (ORRs) in the DRd and Rd arms were 61% and 32%, respectively (HR, 0.36; 95% CI, 0.26–0.50; P <0.0001). AEs were more common in the DRd arm than in the Rd arm (67% vs 40%; P <0.0001). The most common AEs were oedema (24% vs 19%), fatigue (23% vs 21%), constipation (18% vs 12%), and infusion-related reaction (13% vs 8%). The most common grade ≥3 AEs were anaemia (9% vs 5%) and neutropenia (8% vs 7%). The majority of patients showed improvement in baseline laboratory values. Summary/Conclusions: Daratumumab plus lenalidomide in combination with low-dose dexamethasone is highly effective and leads to durable responses in patients with RRMM. The drug is well tolerated with a manageable safety profile, and treatment interruption or discontinuation is rare. The quality of life is maintained during treatment.

P332 LENALIDOMIDE MAINTENANCE VS PLACEBO AFTER STEM CELL TRANSPLANT FOR PATIENTS WITH MULTIPLE MYELOMA: OVERALL SURVIVAL AND PROGRESSION-FREE SURVIVAL AFTER ADJUSTING FOR TREATMENT CROSSOVER IN CALGB

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Background: At a prespecified interim analysis (December 2009), the phase 3 CALGB/ECOG 100104 (Alliance) study results surpassed the prespecified superiority boundary (significantly improved progression-free survival [PFS] for lenalidomide [LEN] maintenance vs placebo [PBO] after SCT) and the majority of PBO arm patients without progressive disease (PD) crossed over to LEN maintenance. An updated analysis (cutoff March 2016) 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, 0.25-1.13). Sensitivity analyses showed consistent results. Updated data will be presented at the meeting.

Summary/Conclusions: Adjusting for the potential diluting effects of crossover reduced median OS and PFS with PBO, and improved the treatment effect in the ITT analyses for OS and PFS for LEN vs PBO maintenance after SCT. The statistical significance of the ITT analyses was maintained throughout.
marrow samples were collected, and minimal residual disease (MRD) was assessed at the time of suspected complete response (CR) and at 3 and 6 months after suspected CR at 3 different sensitivity thresholds (10⁻⁴, 10⁻⁵, and 10⁻⁶) using the ClonoSEQ™ next-generation sequencing-based assay (Adaptive Biotechnologies, Seattle, WA). Additional reflex testing using an anti-idio-
type antibody was used to confirm CRs in cases in which daratumumab inter-
fERENCE with serum M-protein quantitation was suspected in patients with pos-
ible CR.

Results: Patients received a median (range) of 1 (1-11) prior lines of therapy; 55% of patients had received immunomodulatory agents (IMiDs), and 18% had been exposed to lenalidomide. After median follow-up of 17.3 months, DRd sig-
cificantly prolonged PFS compared with Rd alone (median: not reached vs 17.5 months; hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.28-0.50; P<0.0001), with 18-month PFS rates of 76% and 49%, respectively. Responses continued to deepen in the DRd group with longer follow-up, with significantly higher overall response rate (ORR; 93% vs 76%) and rates of very good partial response (vGPR) or better (78% vs 45%) and CR or better (46% vs 20%) with DRd versus Rd alone (P<0.0001 for all), and MRD negativity was associated with prolonged PFS at 10⁻⁵ (Figure 1). Overall survival (OS) data are immature, with 40 (14%) deaths in the DRd group and 56 (20%) deaths in the Rd group (HR, 0.63; 95% CI, 0.42-0.95). Neutropenia was the most common grade 3 or 4 treatment-emergent adverse event (53% with DRd vs 38% with Rd), and no new safety signals were reported with longer follow up. We will present updated efficacy and safety data based on approxi-
ately 25 months follow up at the meeting.

Figure 1.

Summary/Conclusions: DRd significantly improved outcomes compared with Rd alone, including PFS, ORR, depth of response, and MRD-negative rates, with a favorable safety profile that was maintained after longer follow up. These updated data continue to support the use of DRd in patients with RMM who received ≥1 prior therapy.

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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 dex-
amethasone (Vd; CASTOR) or lenalidomide and dexamethasone (Rd; POLL-
UX) significantly prolongs progression-free survival (PFS) with a manageable safety profile compared with either Vd or Rd alone in patients (pts) with RMM.

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 q4w until disease progression or unacceptable toxicity. Pts in POLLUX were treated until progression. Dosing schedules for D (16 mg/kg) were different between CASTOR (qw in Cycles 1-3, q3w for Cycles 4-8, q4w thereafter) and POLL-
UX (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 thrombo-
topenia (45% vs 37% with Vd), fatigue (15% vs 11%), pneumonia (15% vs 17%), and anemia (10% vs 11%). Infusion-related reactions (IRR) occurred in 13 (65%) pts, with 10% having grade 3/4 IRR, but no pts discontinued due to IRR. Median PFS was significantly prolonged with Dvd versus Vd (not reached [NR] vs 11.5 months; hazard ratio [HR], 0.27; 95% CI, 0.13-0.56; P=0.0009), consistent with the overall PFS observed in CAS-
TOR (Figure). Higher overall response rate (ORR; 95% vs 79%) and rates of complete response (CR) or better (25% vs 3%) and very good partial response (vGPR) or better (70% vs 18%) were achieved with Dvd versus Vd, respectively, consistent with the overall population. In the POLLUX study, 29/286 pts in the DRd group and 35/283 pts in the Rd group were aged ≥75 years; the median (range) age for this group of pts was 77 (75-89) and 78 (75-87) years, respectively, with 86% and 91% with an ECOG status ≤1. At a median follow-up of 17.3 months, 10% of pts in the DRd group and 11% in the Rd group discontinued due to TEAEs. Common (≥10%) grade 3/4 TEAEs for DRd were neutropenia (45% vs 31% with Rd), hypokalemia (14% vs 3%), and pneumonia (10% vs 11%). D-associated IRR occurred in 12 (41%) pts in the DRd group, with 4 (14%) pts having grade 3/4 IRR. No pt discontinued DRd because of IRR. Median PFS was significantly prolonged with DRd compared with Rd in the elderly subgroup (NR vs 11.4 months; HR, 0.19; 95% CI, 0.06-0.55; P=0.0007), consistent with the overall PFS observed in POLLUX (Figure 1). ORR was higher with DRd versus Rd (93% vs 77%), and rates of CR or better (52% vs 9%) and vGPR or better (72% vs 41%) were also higher with DRd versus Rd.

Figure 1.

Summary/Conclusions: The results in elderly pts were consistent with those observed in the overall study populations in terms of efficacy. Rates of most common grade 3/4 hematologic TEAEs with DRd were similar to that of the overall populations, and IRR were manageable. This subgroup analysis supports the addition of D to standard-of-care regimens in elderly pts with RMM.
ALL ORAL COMBINATION OF IXAZOMIB PLUS THALIDOMIDE AND DEXAMETHASONE FOR RELAPSED OR REFRACTORY MULTIPLE MYELOMA: INTERIM DATA OF AN ONGOING PHASE II TRIAL

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Background: Ixazomib is a novel, effective oral proteasome inhibitor with a favorable toxicity profile. Recent studies showed significant activity as single agent with dexamethasone and in combination with other agents. The Tourmaline trial showed superior PFS with ixazomib plus lenalidomide and dexamethasone in pts with relapsed or refractory multiple myeloma (RRMM).

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

Methods: Pts with RRMM and one or more prior lines of therapy (TX) with the following criteria were eligible: Measurable disease, ECOG PS ≤2, ANC ≥1000/µL, platelet count ≥500000µL, GFR ≥15mL/min. Treatment regimen: ixazomib (4mg, d 1, 8, 15), thalidomide (100mg/d), and dexamethasone (40mg, d 1, 8, and 15). Pts aged ≥75 years received lower doses of thalidomide (50mg/d) and of dexamethasone (3mg, d 1, 8, 15). Pts were scheduled for 8 cycles followed by ixazomib maintenance therapy (4mg, days 1, 8, 15 of a 28 cycle and 3mg in pts aged ≥75 years) for one year. Primary objective was PFS, and secondary objectives were ORR, OS, impact of cytogenetic risk and of renal impairment, safety and health related QoL.

Results: Sixty-seven of 77 planned pts have been enrolled so far. The following patient characteristics were recorded in the intent-to-treat group (ITT): median age: 67, range 41 to 84 years, ISS stage I: 28, II: 22, III: 16, not known: 1, median number of prior TX lines: 1 (range: 1-8). 9 pts discontinued TX before completion of 2 cycles. Presently, 5 pts are too early for evaluation per protocol (PP). Full documentation of ≥2 cycles is available for 52 pts, with a median number of 4 cycles and a median FU of 7.4 mos. A PR or better was achieved in 33% (63%), nCR: 2 pts (4%), VGPR: 10 (19%), PR: 21 (40%), MR: 2 (4%), yielding a clinical benefit rate (CBR) of 67%. FISH data are available in 43 of the 52 PP pts. Proteinuria >5 gr/day and eGFR <50 ml/min. Treatment regimen: IxaThalDex (4mg, d 1, 8, 15). Pts in 11/18 (61%) with stage I renal (4; 14) and/or dialysis (17) and in 15/25 (60%) with standard risk cytogenetics. Median PFS at the time of analysis was 10.3 mos., p=0.882) vs 10.4 mos., p=0.061) for progression to dialysis in each cohort (Figure 1).

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

EVALUATION OF GROWTH DIFFERENTIATION FACTOR-1 (GDF15) AS A NEW BIOMARKER FOR RENAL OUTCOMES IN DIFFERENT COHORTS OF PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS

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Background: Growth differentiation factor-15 (GDF-15), is a member of the TGF-beta family, and is involved in several pathological conditions, including inflammation, cancer, cardiovascular, pulmonary and renal diseases. Serum GDF-15 levels add prognostic information to conventional prognostic factors, such as NT-proBNP and troponins, in cardiovascular disorders and has also shown to be associated with renal damage and risk of end stage renal disease in patients with diabetes. Increased serum GDF-15 levels have also shown to be correlated with early death and shorter survival independently of other biomarkers and Mayo stage. Because GDF-15 was also associated with renal outcomes we evaluated the prognostic value of GDF-15 levels in two independent cohorts of patients with AL amyloidosis and renal involvement who were treated in two different centers (Pavia Amyloidosis Center and Department of Clinical Therapeutics, Athens).

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

Methods: Circulating levels of GDF-15 were measured by a novel pre-com- mercial 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 biomarker-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.

Figure 1.
Summary/Conclusions: Our study validated and confirmed in two independent cohorts, with differences in their characteristics, the prognostic value of GDF-15, which emerges as a novel biomarker with prognostic implications for different outcomes in patients with AL amyloidosis. Importantly, GDF-15 emerges as a strong biomarker for renal outcomes in patients with AL amyloidosis.

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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, tripleplet 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 during induction. Secondary endpoints included tolerability and toxicity, overall response rate (CR+VGPR+PR) throughout treatment, time to response, PFS, and quality of life (QoL).

Methods: Adult pts with NDMM who were transplant-ineligible were randomized (1:1) to receive oral ixazomib 4.0 mg plus oral cyclophosphamide 300 mg/m² (Arm A) or 400 mg/m² (Arm B) on days 1, 8, 15, and 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 (11q;14; t(1;14); 11q;16); 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 AE (24%); PD (16%); patient withdrawal (3%); or other reasons (10%). Confirmed responses by investigator assessment are shown in the Table 1. Median time to first/best response across arms was 2/4 months. After a median follow-up of 17.9/18.5 months in Arm A/B, median PFS was not reached. Combined PFS at 12/18/24 months was 81%/66%/59% (24-month PFS 64%/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 peripheral neuropathy (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-C30; Global Health Status) was maintained from baseline during the study.

Table 1.

<table>
<thead>
<tr>
<th>Response, n (%)</th>
<th>Arm A (n=33)</th>
<th>Arm B (n=34)</th>
<th>Arm A + Arm B (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>4 (12)</td>
<td>3 (9)</td>
<td>7 (11)</td>
</tr>
<tr>
<td>VGPR</td>
<td>6 (18)</td>
<td>5 (15)</td>
<td>11 (16)</td>
</tr>
<tr>
<td>PR</td>
<td>11 (33)</td>
<td>9 (26)</td>
<td>20 (30)</td>
</tr>
<tr>
<td>SD</td>
<td>13 (39)</td>
<td>16 (47)</td>
<td>29 (43)</td>
</tr>
<tr>
<td>PD</td>
<td>2 (6)</td>
<td>2 (6)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>36 (107)</td>
<td>27 (78)</td>
<td>63 (94)</td>
</tr>
<tr>
<td>CR+VGPR+PR</td>
<td>10 (30)</td>
<td>8 (24)</td>
<td>18 (27)</td>
</tr>
</tbody>
</table>

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-orall triplet including a PI and alkylator, provide evidence of clinical efficacy with a manageable safety profile. With a median follow-up of ~18 months, median PFS was not reached and outcomes appear comparable to other regimens in elderly transplant-ineligible pts with NDMM. The preferred cyclophosphamide dose for ICd phase 3 studies is 300 mg/m², based on the similar PFS, higher response rate, and numerically lower rate of AEs vs 400mg/m². Updated PFS results will be presented at the meeting.

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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 9 mg/m² and P 60 mg/m² (days 1–4) in 42-day cycles (max 9 cycles). In phase 2, an expansion cohort was enrolled at the RP2D. On all arms, after MP induction, pts could receive maintenance with single-agent ixazomib (days 1, 8, 15; 28-day cycles).

Table 1.

<table>
<thead>
<tr>
<th>Response, n (%)</th>
<th>Arm A (n=11)</th>
<th>Arm B (n=34)</th>
<th>Arm C (n=11)</th>
<th>Arm D (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>2 (18)</td>
<td>4 (12)</td>
<td>2 (18)</td>
<td>0</td>
</tr>
<tr>
<td>VGPR</td>
<td>5 (45)</td>
<td>6 (18)</td>
<td>3 (27)</td>
<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>4 (36)</td>
<td>14 (41)</td>
<td>5 (45)</td>
<td>2 (40)</td>
</tr>
<tr>
<td>SD</td>
<td>8 (73)</td>
<td>9 (26)</td>
<td>4 (36)</td>
<td>5 (100)</td>
</tr>
<tr>
<td>PD</td>
<td>1 (9)</td>
<td>1 (3)</td>
<td>1 (9)</td>
<td>0</td>
</tr>
<tr>
<td>Overall response rate</td>
<td>7 (63.6)</td>
<td>15 (44)</td>
<td>7 (63.6)</td>
<td>2 (40)</td>
</tr>
</tbody>
</table>

Results: 61 pts were enrolled, 11, 34, 11, and 5 to Arms A, B, C, and D (median age 74 yrs; 31% ISS stage III, 56% creatinine clearance ≤60 mL/min). Among
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 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.

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 ≥PR 73%, ≥VGPR 38% and ≥CR 11%, not significantly different in unfit versus frail patients. Response after 6 cycles was ≥PR 69%, ≥VGPR 35% and ≥CR 2%. Median progression free survival (PFS) was 17 months: 20 for unfit and 16 months for frail patients (p=0.13). Overall survival at 18 months was 76%: 89% for unfit and 72% for frail patients (p=0.22). Frail patients were found to have significantly less grip strength and lower walking speed as compared to unfit patients (Table 1).

Table 1.
walking speed and 12% of patients with low grip strength, were not frail. Therefore, functional assessments will hopefully be complementary to the IMWG frailty score in guiding future therapy in unfit and frail patients.

**Material and Methods:**

**Chemotherapy before and after heart transplantation for patients with advanced cardiac amyloidosis**

**Results:**

- **Survival rates for patients with light-chain (AL) amyloidosis are gravely reduced by advanced cardiac involvement at Mayo cardiac stage IV with a median survival of 6.5 months. High-dose Melphalan (HDM) and autologous stem cell transplantation (ASCT) or other intensive chemotherapy regimens cannot be applied to these patients due to the high risk of therapy-related mortality.**

**Aims:**

- Our aim was to examine the cases of cardiac AL patients treated with heart transplantation (HTx) at our center and to evaluate the clinical outcome of this treatment approach.

**Methods:**

- Data from 41 patients (21m, 20f) suffering from cardiac AL who were treated in our hospital between 2002 and 2017 were retrospectively analyzed. All patients were high-urgency listed for orthotopic HTx due to poor prospective of survival. Until 2009, 11 patients were listed, 8 of them with multiple organ involvement. Thereafter, we excluded patients with multiple organ involvement. All data are derived as medians with range or absolute numbers. Survival curves were calculated using the Kaplan-Meier method.

**Results:**

- Median age was 51 years (35-63) at diagnosis. Amyloidogenic lambda light-chains (LC) were detected in 35 and kappa light-chains in 13 patients. Median dFLC was 331 (69 - 2752) and median plasma cells in bone marrow were 13% (5-35). Median NT-proBNP was 6.332 ng/l (1.500 - 53.194), median cTNT 0.11 µg/l (0.01 - 0.52) and median hsTNT was 60 ng/l (28-448) at diagnosis. Median NYHA stage was 3 (2-3) and median MACC 2004 stage was 3 (2-3). Serum creatinine was at 91 (74-106) µmol/l (0.8-1.3), creatinine clearance at 89 (41-106) ml/min. Patients stayed on the high-urgency waiting list for a median of 26 (range 3-54) before 2009, and a median of 64 days (8-259) after 2009. 35 patients were treated with chemotherapy prior to HTx (mostly dex/vor/LoB) to reduce dFLC during the waiting time. Eight patients died before receiving HTx with a median survival (start point: HU listing) of 26 days (6-177). With a median of 5 months after HTx (4-29), 18 patients received ASCT. 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 treated pts, n=29; 2 patients have not finished treatment yet), very good partial remission (VGR) in 7 patients (21%) and partial remission (PR) in 7 patients (24%). Overall, 25 patients died. Cause of death was either progression of AL (N=16), sepsis (n=4), heart transplant rejection (n=3) or other (n=2). Patients that underwent HTx had a median survival of 46 months (2-177, 1-year survival: 77%).

**Summary/Conclusions:**

- HTx improves symptoms but 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 (VGR or better) and consecutively a favorable survival probability with a median OS of nearly 6 years in our series.
or unacceptable toxicity. Supportive care was allowed; thrombophlebitis 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, 1-10) prior anti-myeloma therapies. All pts had prior treatment with LEN (100%) and nearly all with BORT (97.5%). Median relative dose intensity of POM was 0.94 in both cohorts A and B, and 0.99 in cohort C. ORR was 39.4%, 29.4%, and 14.3% in cohorts A, B, and C respectively. PFS and OS results are presented in the Table 1. Grade 3/4 anemia and thrombocytopenia occurred more frequently in cohort C, likely due to severe RI requiring dialysis (Table 1). AEs leading to dose reductions were 18.2%, 14.7%, and 14.3% in cohorts A, B, and C respectively.

Table 1.

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.

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ASSESSMENT OF MOBILIZATION COST FOR MULTIPLE MYELOMA USING 2 DIFFERENT STRATEGIES: HIGH-DOSE CYCLOPHOSPHAMIDE VERSUS PLERIXAFOR. ON BEHALF OF IFM


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Background: Treatment with autologous transplantation (ASCT) remains the standard of care upfront for Multiple Myeloma patients considered eligible for transplant. Peripheral blood stem cell (PBSC) collection, also called mobilisation, is needed prior to ASCT. The optimal methodology for mobilizing PBSC has yet to be defined, with either G-CSF alone, also called steady state procedure, or use of Plerixafor, a CXCR4 antagonist (Mozobil®)-G-CSF or high dose cyclophosphamide (usually administered at a dose of 1.5 to 6g/m² IV for 1 day) is commonly used. The most recent induction regimens, and the 2 latter have demonstrated similar PBSC collection rates. Because of the intense competition for hospital resources and the staff required to manage patients preparing for mobilization and transplantation, it is important to quantify the total impact of mobilization on staff resource and ward budget at the hospital.

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

Methods: This is an observational cohort database analysis of 112 consecutive patients with MM treated upfront with ASCT between 2009 and 2013 and that had been mobilized with either high dose cyclophosphamide or plerixafor from 15 IFM centers. Patients must have successfully underwent ASCT. This study was not aimed at evaluating the suitability or advisability of one therapy versus another. A cost-consequences analysis of the different regimens of mobilization was performed. ECONOTE-023 (NCT01953692) is a multicohort phase 1b study of pembrolizumab monotherapy in patients with hematologic malignancies; results are reported for patients with RRMM.
DISCUSSION.

The point of view of the French Public Health System is adopted for this study. Monetary values for 2012 euros prices will be used for all components.

Results: Median (range) age was 59.5 (24-72), sex ratio was 1.5, ISS 3 was 26% in either group, all patients were collected to allow the number of graft requested by the hematologist of reference. The median CD34 collected was 8.9 (4-30) for HD cyclophosphamide and 5.3 (2-15) for plerixafor. The analysis demonstrated that days in the hospital were the primary cause for cost difference across the 2 mobilization techniques. In that regards, plerixafor appeared very cost effective compared to HD cyclophosphamide. All data will be updated at EHA 2017 including cost comparison.

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.

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A STUDY OF UTILITY OR FUTILITY OF PERFORMING SKELETAL SURVEYS IN PARAPROTEINAEMIA: A MULTICENTER EXPERIENCE

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Background: Recent International Myeloma Working Group (IMWG) guidelines recommend that conventional skeletal surveys should be supplanted by low dose whole body computed tomography (CT), whole body magnetic resonance imaging (MRI) and or 18fluoro-deoxyglucose (18F-FDG/CT) PET. However, resource, funding and radiology capacity issues, have posed significant challenges to implementing these recommendations. The risk of progression of Monoclonal Gammopathy of Undetermined Significance (MGUS) to a neoplastic plasma cell disorder is approximately 1% per year2 and even lower in low risk MGUS. It is thus not necessary to perform imaging in unselected MGUS patients.

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

To decide if a rational clinic–biochemical algorithm could be used to reduce the number of imaging requests, thereby avoiding unnecessary radiation exposure, and make a possible switch to modern imaging methods cost effective.

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

A pragmatic algorithm was developed and applied to see if the requests were justified and could have been safely reduced. (Figure 1).

Results: Of the 397 analyzable skeletal surveys performed, 266 were on myeloma, 81 for MUGS, 48 were for non-paraprotein related indications. Of the 266 myelomas, 30% of skeletal surveys were reported as positive according to IMWG criteria3. 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.

Figure 1.

Summary/Conclusions: - Skeletal survey has very limited role in investigation of paraproteinemia and should be abandoned. - Our pragmatic clinic-biochemical imaging algorithm reduced imaging requests significantly (24%) allowing the preferred imaging modalities to be performed productively in a cost effective way in face of ever increasing health care cost and demands.

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SERUM FLC MEASUREMENTS COMPLEMENT BONE MARROW ASSESSMENT TO DETERMINE PROGNOSIS IN MYELOMA PATIENTS ACHIEVING DEEP RESPONSES

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Summary/Conclusions: - Serum FLC measurements complement bone marrow assessment to determine prognosis in myeloma patients achieving deep responses.

Figure 1.
Background: In multiple myeloma (MM), abnormal serum free light chain ratios (FLCr) after therapy associate with poor prognosis, independent of depth of response. However the value of FLCr in the context of minimal residual disease (MRD) remains unclear. A proportion of MRD-negative patients experience early relapses and conversely, some MRD-positive patients can endure long-term survival; which may result from improved immunosurveillance following normal plasma-cell recovery.

Aims: We hypothesised that serum FLC levels and ratios add clinical value at the time of MRD assessment.

Methods: The study included 275 intact immunoglobulin MM patients from the IFM2009 clinical trial who achieved at least a very good partial response (VGPR) after consolidation therapy. Median PFS from the end of consolidation was 38.3 months; median OS was not reached. Serum FLCs were measured using Freelite immunoassays (The Binding Site). Normal range for k/l FLCr was 0.26-1.25. We defined immunosuppression as levels of both the uninvolved (polyclonal) FLC+uninvolved heavy+light chain (HLC; measured with Helyxite) 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 363 MRD-negative patients, 37/196 (19%) had abnormal FLCr, 2/196 (1%) had elevated iFLC with immunosuppression identified in 23/196 (12%), Median PFS for MRD-negative patients was not reached; however both an abnormal FLCr (median PFS: 31.4 months; p<0.001) and immunosuppression (median PFS: 31.4 months; p=0.005) identified a group of patients with poorer outcomes. On the other hand, median PFS for MRD-positive patients was 21.3 months; 42(63%) of these patients had abnormal FLCr and dismal outcomes (median PFS 12.6 vs 30.7 months for abnormal vs normal FLCr, respectively; p=0.004). Absolute FLC measurements did not reach statistical significance for PFS in these patients.

Summary/Conclusions: Serum FLC measurements in combination with low-sensitivity MFC bone marrow assessment at the end of consolidation therapy render the most powerful prognostic information in MM patients achieving deep responses. In those where disease is no longer detected using MFC, abnormal FLCr confer poor prognosis, which may partly be due to inefficient immune recovery. Absolute FLC measurements were not informative, supporting the rationale of evaluating biomarkers of the tumour and immune system recovery. Our results warrant further studies to validate the clinical utility of FLC measurements in combination with next-generation (8-colours) flow cytometry.

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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) (Avet-Loiseau, Leukemia, 2013). Historically, t(11;14) has been associated with standard-risk multiple myeloma (MM) and generally favorable outcomes (Avet-Loiseau, Leukemia, 2013). However, some recent retrospective analyses 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 (14/14), 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 insignificant (P=0.004).

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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IN JUVENILE MYELOMONOCYTIC LEUKEMIA

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

Background: Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloproliferative disorder of early childhood. While some cases show spontaneous remission, allelogenic 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 JMML. It is caused by genetic alterations in \( CBL, KRAS, NFI, NRAS, \) or \( PTPN11 \) in about 90% of patients. So far, there is no clear understanding of how RAS pathway mutations relate to the heterogeneous disease biology and variable clinical outcome seen in JMML patients. As a consequence, established clinical and genetic markers fail to fully represent the observed disease heterogeneity. 

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 JMML 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 JMML (n=130) or Noonan syndrome associated myeloproliferative disorders (n=18) in the validation cohort.

Results: Systematic DNA methylation analysis of JMML samples identified three subgroups with low, intermediate and high methylation levels (LM, IM, and HM). Detailed analysis of the validation cohort not excluding the Noonan patients identified an association of methylation groups with clinical features. The HM subgroup (n=41) 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/nl), and 66% carried somatic mutations. Within the whole cohort of 781 SMF patients, 376 had cytogenetic abnormalities. In addition, prognostic relevance of different cytogenetic patterns is investigated.

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

Results: Within the whole cohort of 781 SMF patients, 376 had cytogenetic data. Cytogenetic abnormalities were reported in 128 (34.1%) cases: 72 (60%) were sole, 22 (18.3%) double, 26 (21.7%) complex, 11 (9.2%) MK (all included in complex karyotype) and eight unbalanced. The median number of individual abnormalities were 20p (25%), 13q (20.8%), +8 (8.3%) and +9 (5.6%). Patients with post-PV MF had significantly higher frequency of abnormal karyotypes than those with post-ET MF (P<0.012). Chromosomal abnormalities did not cluster differently among the different genotypes (JAK2, CALR, MPL and triple negativity). Abnormal karyotype was significantly associated with lower platelet count (P=0.004), larger spleen size (P=0.016), higher circulating blasts (P<0.001) and presence of constitutional symptoms (P=0.014) at the time of SMF diagnosis. Within abnormal karyotypes, we found that patients with MK tended to have lower platelet count (P=0.04) with respect to those with sole aberrations. Overall survival in patients with pure MK was significantly worse compared to patients with only structural abnormalities (P=0.012), even adjusting for SMF diagnosis type (P=0.02). When assessing OS according to different abnormalities, we found that patients with MK have inferior OS than those with sole abnormality (P<0.0001).
was 70 years (21-89). Median (range) values for leukocytes, neutrophils, hemo-
globin, platelets and bone marrow blast percentage at the time of sample collection 
for sequencing were 13.4 (1-179) x 10^9/L, 7.9 (0.4-152.4) x 10^9/L, 9.1 (3.1-15) 
g/dL, 123 (6-1168) x 10^9/L and 2% (0-17), respectively. On univariate analysis 
(n=97), only the presence of EZH2 and ZRSR2 mutations were associated with 
trends towards statistical significance for survival. Mutated EZH2 adversely 
affected survival (p=0.089) and mutated ZRSR2 resulted in a comparable 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 
combine) were statistically significantly associated with inferior and superior 
overall 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) and cell signaling molecules (JAK2, KRAS, NRAS, BRAF), thylases (FABP3, 
FPN1), the transcription factor RUNX1, and the nuclear receptor SETBP1 were at frequencies ≥10%. Although the analysis is limited by small numbers, EZH2 mutations were independently associated with poor 
overall survival. This represents the largest cohort of patients with MDS/MPN-U inter-
rogated for mutations in multiple genes to date.

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MUTATIONAL LANDSCAPE OF MYELODYSPLASTIC SYNDROME/ 
MYELOPROLIFERATIVE NEOPLASM - UNCLASSIFIABLE
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et al. Blood 2014). The International 
Prognostic Scoring System (IPSS) for MDS (Greenberg et al. Blood 1997) dis-
criminated amongst prognostically distinct categories in that cohort, while nei-
ther 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-institu-
landscape of MDS/MPN-U is limited to one report on the frequency of SETBP1 
mutations (8.3%, Meggendorfer et al. Leukemia 2013).

Aims: To describe the mutational landscape of MDS/MPN-U using targeted 
multi-gene sequencing.
Methods: Targeted sequencing was performed on DNA from 97 patients with MDS/MPN-U (diagnosed per WHO 2008 criteria but excluding refractory anemia with ringed sideroblasts and thrombocytemia) seen across 4 US institutions (MDACC, 43; Cleveland Clinic, 29; Moffit Cancer Center, 16; Vanderbilt Univer-
sity, 9). Gene panels varied among institutions, with 20 genes (ASXL1, CBL, 
DNMT3A, ETV6, EZH2, IDH1, IDH2, JAK2, KIT, NPM1, NRAS, PHF6, 
RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, ZRSR2) in common.

Results: Mutational frequencies for the 20 genes tested in all 97 patients were 
as follows: TET2, 28%; ASXL1, 27%; JAK2, 25%; SRSF2, 22%; EZH2, 15%; 
SF3B1, 12%; RUNX1, 12%; ZRSR2, 11%; SETBP1, 11%; U2AF1, 11%; NRAS, 10%; 
DNMT3A, 9%; TP53, 8%; CBL, 4%; ETV6, 4%; NPM1, 4%; IDH2, 2%; 
KIT, 2%; PHF6, 1% and IDH1, 0%. In addition, the frequency of mutations in 
eten 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=52); 
TLK, 8% (n=72); CEBPA, 4% (n=73); KRAS, 4% (n=81); PTPN11, 4% (n=82) and 
FLT3, 2% (n=82). Median survival for the whole cohort (n=97) was 
12.4 months (range, 1-173). The 43 MDACC patients in this analysis were 
included in the cohort of 85 previously reported by DiNardo et al. Median age

Figure 1.
Summary/Conclusions: Abnormal karyotype was found in 34.1% of SMF 
patients at diagnosis and was over-represented in post-PMF MF. No different 
distribution was detected among genotypes. Abnormal karyotype was associ-
ated with lower platelet count, larger spleenomegaly, higher circulating blast 
cells and presence of constitutional symptoms. Concerning outcome, the pres-
ence of abnormal karyotype implied inferior survival and, among subtypes, MK 
remained the most powerful predictor.

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GENOME WIDE DNA METHYLATION PROFILING IS PREDICTIVE OF OUTCOME IN JUVENILE MYELOMONOCYTIC LEUKAEMIA
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Background: Juvenile myelomonocytic leukemia (JMML) is a myeloprolifera-
tive disorder of childhood that is initiated by mutations in the Ras pathway.
In outcomes this disease vary dramatically from resolution with minimal or no 
therapy to relapse despite hematopoietic stem cell transplantation. Identifying 
biomarkers to distinguish patients with aggressive disease courses from those 
who can receive minimal therapy remains a priority for clinicians.

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 pre-
dicting 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-character-
ized patients with JMML enriched for those who experienced spontaneous 
resolution without chemotherapy. A separate cohort of 40 patients with JMML 
was recruited 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 hyperme-
thylated 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 popula-
tions and unsorted monocellular cells. Unsupervised clustering of the dis-
covery cohort based on the most highly variable CpG sites (top 0.5% ranked 
significance) led to the identification of three clusters (top 6%). 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 interme-
diate 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 into 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. Remarkably, 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 NSS PROFILE THE BEST PROGNOSTIC BIOMARKER?

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Background: Leukemic transformation occurs in 8% to 23% of myelofibrosis patients in the first 10 years after diagnosis and in 4% to 8% of polycythemia vera and essential thrombocythemia patients within 18 years of diagnosis and is usually fatal.

Aims: We retrospectively analyzed the survival outcome of patients with myeloproliferative neoplasms (MPNs) who progressed to acute myeloid leukemia (AML) based on the treatments received, response, different prognosis groups according to the (ELN) and based on a next-generation DNA sequencing profile (NGS).

Methods: A total of 72 patients diagnosed in our institute with IAML secondary to MPNs between 2000 and 2016 were retrospectively analyzed. NGS was performed in 44 cases. Mutations found by NGS were classified according three different categories of interest (Tumors suppressor (TP53), ADHPR44, and epigenetic (DNMT3A, EZH2,H3K27/12/1/ASHXL) and alternative splicing (SRS2F2/2A2AF1, ZRS2R,PRP8,F3B3)) and three groups were determined: Group A: patients without altered cellular function; Group B: patient with one altered function; Group C: patients with more than one altered functions. AML treatment response was evaluated according Mascarenhas’ proposed criteria for response assessment of AML secondary to MPNs. Overall survival (OS) was calculated according the different treatments, treatment response and NGS profiles.

Results: 72 patients who developed AML secondary to MPNs were included in the study: 43.6% (N=31) had prior ET, 25% (N=18) PV, 20.8% (N=15) PMF and 11.1% (N=8) secondary myelofibrosis. The median age at AML transformation was 70 (range: 38-89) years. The median time to AML transformation from MPNs diagnosis was 108 months (range: 2.4-408). Among these 72 AML, 5.6% (N=4) belonged to the favorable risk category according to ELN 2017. 13.9% (N=10) belonged to the intermediate risk category and 55.6% (N=40) to the adverse risk category. 45.8% (N=33) patients were treated with intensive chemotherapy (IC), 15.3% (N=11) with azacitidine (AZA) and 38.9 (N=28) with supportive care (BSC). Median OS was 4.5 months (range, 0.1-65), with no significant difference between the three ELN 2017 risk categories (respectively 2.5 months (range: 1-9), 5.5 months (range: 1-60) and 5 months (range: 1-36) in the favorable, intermediate and adverse risk categories). Patients who received IC (p<0.01) or AZA (p<0.05) have a significant better OS (median OS of 7 months (range: 0.5-65) and 8.5 months (range: 3-24) respectively) than patients who received BSC (median OS of 2 months, range: 0.1-36). However, there was not a significant difference between the IC and HMA groups (p=0.44). 7 Patients in Complete Cytogenetic Response (CCR) or Acute Leukemia Response-Complete (ALR-C) received an alloSCT had a better median OS than the 9 patients who did not (23 vs 6.5 months, p=0.063). Patients with group A and B NGS profiles have a significant better median OS (respectively 14 and 8 months) than Group C (3 months) (p<0.05).

Summary/Conclusions: Our results confirm the poor outcome of patients with secondary AML treated with IC and suggest that AZA provides comparable OS. ELN2017 risk stratification predicted poorly patients outcome although a NGS-based classification performed better.

INCIDENCE AND OUTCOME OF SECONDARY NON HEMATOLOGICAL CANCERS IN ADULT PATIENTS WITH MASTOCYTOSIS

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Background: Mastocytosis is a clonal disease characterized by heterogeneous manifestations and a normal life expectancy in the majority of cases. In such a condition, it is important to ascertain if other diseases, and particularly solid malignancies, can worsen the prognosis.

Aims: To assess incidence and outcome of secondary primary malignancies (SPM) in adult mastocytosis patients.

Methods: We performed a retrospective analysis of 826 adult (>18 years at diagnosis) mastocytosis patients diagnosed and regularly followed in 6 Italian Institutions. SPM were defined as de novo cancers diagnosed after mastocytosis. 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 Hematological Neoplasm (AHN). Standardized Incidence Ratio (SIR) was calculated as the ratio between the observed cases in our cohort and the expected cases in the sex- and age-matched general Italian population in the same time period (these data were retrieved from http://www.registrni-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), Smoldering 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 39-72). The median time from the diagnosis of mastocytosis 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 1.46, 95%CI: 0.9-2.4). We found a clearly increased risk for melanoma (n=8, SIR 15.9, 95%CI: 7.9-31.9) and thyroid cancer (n=3, SIR 9, 95%CI: 2.9-27.9) while a non-significant increased risk was found for prostate cancer in males (n=5, SIR 2.06, 95%CI: 0.8-4.9) and breast cancer in females (n=3, SIR 1.7, 95%CI: 0.5-5.3). All the other malignancies were sporadic (one or two cases for each cancer type) and comparison to the general population was not significant. The death rate in patients with SPM was 14.7% with a median overall survival (OS) from SPM diagnosis of 38 months. OS was significantly inferior in patients with SPM as compared to patients without secondary neoplasia (5-year OS 77.6% vs 93.7% respectively, p<0.01) (Figure 1).

Figure 1.

Summary/Conclusions: Patients with mastocytosis may have a significantly higher risk of developing a secondary non hematological cancer as compared to the matched general population. Careful follow-up of these patients is warranted as the rate of malignancies may increase over time and reduce life expectancy.
Background: Colony stimulating factor 3 receptor gene (CSF3R)-mutated chronic neutrophilic leukemia (CNL) is a rare chronic myeloproliferative neoplasm. There is the limited information on the clinical course of CNL.

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

Methods: A retrospective study was conducted to assess natural history and identify risk factor(s) for survival in patients with CSF3R-mutated CNL. Survival analysis was performed by the Kaplan-Meier method taking the interval from the date of diagnosis to death or last contact. The log-rank test was used to compare survival data. Cox regression model was used for multivariable analysis.

Results: Data of 47 patients with CSF3R-mutated CNL were collected and analyzed. 35 (76%) patients were male. Median age was 62 years (range: 16-92 years). At diagnosis, 17 (36%) patients had fatigue, 2 (4%) had a fever, 8 (17%) experienced diarrhea or abdominal discomfort, 20 (43%) were asymptomatic and leukocytosis had been mostly an incidental laboratory finding. 20 (43%) patients had palpable splenomegaly, and 4 (9%), palpable hepatomegaly. PB parameters, median and (range), were WBC 42.4×10^9/L (14.1-180×10^9/L), platelets 165×10^9/L (71-537), blast percentage 0% (0-10), neutrophil percentage 82% (70-99). The median of blast cells in bone marrow were 1% (range, 0-12%). 46 (98%) patients were in the chronic phase and 1 (2%) in the accelerated phase at diagnosis. Most of the CSF3R mutations was T618I (n=45, 96%), others were T568M (n=1, 2%), 5q deletion (n=1, 2%), patients and 41 (87.2%) patients were screened for ASXL1 and SETBP1 mutations, respectively. 21 (61.8%) patients harbored ASXL1 mutation and 22 (53.7%) harbored SETBP1 mutation. All patients were BCR-ABL1, PDGFR and FGR mutation negative, 2 were CALR mutation and JAK2V617F mutation positive, respectively. Hydroxyurea was the most frequently used therapy (n=48). Other therapies included interferon-α (n=7), hypomethylating agents (n=4), thalidomide (n=2), ruxolitinib (n=1), imatinib (n=3), dasatinib (n=1), chemotheraphy (n=6), and transplant (n=2). With a median follow up of 17 months (range, 2-103 months), 7 patients progressed to blast crisis or acute myeloid leukemia (n=6) or myelodysplastic syndrome (n=1), 17 patients died. Survival rate at 30 months was 55%. Median survival was 39 months (95% CI 8.5-69.5). Multivariate analysis showed that WBC >40×10^9/L (HR=3.26, 95% CI 1.14-9.30, p=0.027) was the sole risk factor for survival. However, SETBP1 or ASXL1 mutation was not associated with survival.

Summary/Conclusions: High WBC count was independently predictive of shortened survival in patients with CSF3R-mutated CNL.

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CLINICAL PHENOTYPE AND OUTCOME OF ESSENTIAL THROMBOCYTHEMIA AND PREFRIBROTIC MYELOFIBROSIS DIAGNOSED ACCORDING TO THE REVISED 2016 WHO DIAGNOSTIC CRITERIA

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Background: The World Health Organization (WHO) classification system for myeloid neoplasms was recently revised in 2016. The revised WHO criteria underlines the importance of differentiating prefibrotic PMF (prePMF) from “true” essential thrombocythemia (ET) as these two entities have different clinical outcomes. For these reasons, standardization of morphologic findings in the bone marrow biopsy and an explicit definition of minor clinical criteria for the diagnosis of prePMF have been added (anemia, leukocytosis >11 x10^9/L, palpable spleen, increased LDH) to 2008 WHO criteria.

Aims: To compare the clinical phenotype at diagnosis and the outcome of ET and prePMF diagnosed according to the new 2016 WHO criteria.

Methods: We identified in our database all patients affected with ET, prePMF and PMF diagnosed according to 2008 WHO criteria who satisfied these two requirements: a bone marrow fibrosis grade 0-1 at diagnosis and at least one DNA sample to define the mutational status. Firstly, the bone marrow morphology of all 404 identified patients was reviewed by an expert pathologist. Then, we reclassified patients according to the new 2016 WHO criteria as follows: patient with ET morphology were classified as ET, patients with PMF morphology and at least one clinical criteria (leukocytosis, anemia, increased LDH, splenomegaly) were classified as prePMF, patients with PMF morphology but without clinical criteria were classified as myeloproliferative neoplasms unclassifiable (MPNu).

Results: According to the new criteria our cohort included 269 patients with ET, 109 patients with prePMF and 62 with MPNu. By comparing clinical phenotype at diagnosis in prePMF, MPNu, and ET respectively, we observed that prePMF showed higher leukocyte count, lower hemoglobin levels, higher platelet count, higher LDH values, higher number of circulating CD34-positive cells, and showed more frequently splenomegaly (Table 1). The higher frequency of CALR mutations in prePMF compared to ET might contribute to the high level of platelet count observed in prePMF. ET and MPNu did not differ in terms of leukocyte count, hemoglobin, platelet count, LDH, circulating CD34-positive cells and splenomegaly (Table 1). The 26 patients with MPNu were not further considered in the analysis of disease complications and overall survival due to the low number. PrePMF patients had lower overall survival (overall survival at 10 years 86.4% vs 96.6%, P < 0.001) and a trend to a higher incidence of leukemic evolution (cumulative incidence of acute myeloid leukemia at 10 years 2.3% vs 1.9%, P = 0.067) compared to ET patients, while they did not differ in terms of thrombotic complications (cumulative incidence of thrombosis at 10 years 18.5% vs 18%, P = 0.96). Finally, we analyzed the subgroup of “old” ET diagnosed according to 2008 WHO criteria. Of 358 “old” ET, 268 were reclassified as ET, 25 as MPNu and 65 as prePMF. The “old” ET reclassified as prePMF had a higher risk of overt myelofibrotic evolution compared to the “old” ET reclassified as ET (cumulative incidence of overt myelofibrosis at 10 years 9.7% vs 0%, P = 0.03).

Table 1.

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.
Background: The minimal effective treatment in Essential Thrombocythemia (ET) patients is tailored mainly on the basis of thrombotic risk scores (primarily non nocere). The Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) is based on different combinations of Age >60 yrs (Age >60), JAK2 V617F mutation (JAK2+) and Prior Thrombosis (PrTh+). According to the R-IPSET-Th score, the patients were divided in 4 thrombotic risk groups: Very Low Risk (VLR: Age <60, absence of JAK2 mutations, no PrTh+), Low risk (LR: only JAK2+, or Age >60 with JAK2+), Intermediate Risk (IR: only Age >60), High Risk (HR: PrTh+, or Age >60 with JAK2+). The rates (n and%, n/100 pt-yrs) of first thrombotic events during the follow-up (Th-FUP) were reported for each risk group. The thrombosis free survival (TFS, time from diagnosis to the first thrombosis) was determined for each risk group (Kaplan Meier analysis), and the curves were compared with the log-rank test.

Methods: The web-based Registro Italiano Trombocitemie (RIT) recruited since 2005 patients with thrombocytopenic bcr/abl negative chronic myeloproliferative neoplasms (MPN). ET patients (reclassified according to WHO 2016 criteria) with complete information (characteristics at diagnosis, antithrombotic and/or cytoreductive treatment, date and description of thrombotic events during the follow-up) were considered for this analysis. According to the R-IPSET-Th score, the patients were divided in 4 thrombotic risk groups: Very Low Risk (VLR: Age <60, absence of JAK2 mutations, no PrTh+), Low risk (LR: only JAK2+, or Age >60 with JAK2+), Intermediate Risk (IR: only Age >60), High Risk (HR: PrTh+, or Age >60 with JAK2+).

Overall, 734 ET patients were analyzed (females 62%). Data at diagnosis were: Age >60 in 286 (39%), JAK2+ in 417 (57%), and PrTh in 126 (17%). The Th-FUP were reported for each risk group. The thrombosis free survival (TFS) progressively decreased (p <0.001): in VLR (n=15, 8%), in LR (n=20, 10%), in IR (n=12, 15%), in HR (n=56, 21%). The Th-FUP/100 pt-yrs increased (p<0.01) as follows: 0.60%, 0.79%, 1.61%, and 1.91%, respectively. TFS progressively decreased (p <0.001) from VLR group to HR group (Figure 1). In detail, the probability of TFS was 0.98, 0.97, 0.94, 0.88 at 5 years, and 0.85, 0.87, 0.78, 0.54 at 20 years. The patient stratification according to the R-IPSET-Th and the IPSET-Th scores showed a concordance of 0.82 (Harrell C index).

Results:}

Summary/Conclusions: In this study of the Registro Italiano Trombocitemie (RIT), we confirmed that the Revised International Prognostic Score for Thrombosis in ET (R-IPSET-Th) separated ET patients in 4 groups with increasing risk of thrombosis during the follow-up (p <0.001). According to the R-IPSET-Th score, it seems to have occurred in this cohort of ET patients (anti-platelets in almost all cases), and cytoreduction in around 2/3 of VLR and LR cases, probably because other adjunctive risk factors have been considered.

Figure 1.

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 index.

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 male 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 MF symptoms (MFSAF symptom scores at the same visit). Correlations of Baseline Biomarkers and Symptoms. Total symptom score (TSS) significantly statistically significantly (p<0.05) correlated with 20 BMKs at baseline (Table 1). For individual symptoms, spleen-related symptoms appeared to statistically and significantly correlate more frequently with BMKs at baseline: abdominal discomfort (23 BMKs), feeling full (20 BMKs), and pain under left ribs (19 BMKs). Night sweats, itchiness, and bone or muscle pain significantly correlated with 15, 14, and 10 BMKs each. The BMKs with the strongest correlations (absolute Spearman correlation of at least 0.20 with p<0.001) with at least one symptom included APOA1, EPO, FERRITIN, MIP1A, and PSAF. Associations with Symptoms+TSS, 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 having biomarker values paired with MFSAF symptom scores at the same visit. Correlations of Baseline Biomarkers and Symptoms.

Table 1.

Summary/Conclusions: In this study of the Registro Italiano Trombocitemie (RIT), we confirmed that the Revised International Prognostic Score for Thrombosis in ET-et (R-IPSET-Th) separated ET patients in 4 groups with increasing risk of thrombosis during the follow-up (p <0.001). According to the R-IPSET-Th score, it seems to have occurred in this cohort of ET patients (anti-platelets in almost all cases), and cytoreduction in around 2/3 of VLR and LR cases, probably because other adjunctive risk factors have been considered.
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 leucocyte inclusion bodies, normal ristocetin cofactor test, normal platelet aggregation and normal platelet GPIib/IX expression. Western blot showed lost band of αIIbβ3 in patients compared with normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient's platelets. Hematological examination revealed that there was no difference in the total amount of αIIbβ3 among the affected and unaffected members and normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient's platelets as reflected by the spontaneous binding of PAC-1 antibody. Immuno-fluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIb/β3 p.T746del-expressing cells. Immunofluorescence staining using CHO cells with fluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIb/β3 p.T746del-expressing cells. Western blot showed increased band of αIIbβ3 in patients compared with normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient's platelets as reflected by the spontaneous binding of PAC-1 antibody. Immuno-fluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIb/β3 p.T746del-expressing cells. Western blot showed increased band of αIIbβ3 in patients compared with normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient's platelets as reflected by the spontaneous binding of PAC-1 antibody. Immuno-fluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIb/β3 p.T746del-expressing cells. Western blot showed increased band of αIIbβ3 in patients compared with normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient's platelets as reflected by the spontaneous binding of PAC-1 antibody. 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Immuno-fluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIb/β3 p.T746del-expressing cells. Western blot showed increased band of αIIbβ3 in patients compared with normal controls. FCM showed a constitutive activation of αIIbβ3 on the patient's platelets as reflected by the spontaneous binding of PAC-1 antibody. Immuno-fluorescence staining using CHO cells showed membrane localization of αIIbβ3 in wild-type αIIb/β3-expressing cells and cytoplasmic localization in αIIb/β3 p.T746del-expressing cells. Western blot showed increased band of αIIbβ3 in patients compared with normal controls.

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 unclips the highly conserved membrane proximal complex of αIIb and β3 cytoplasmic tails and renders the activated form. Activation of αIIbβ3 leads to phosphorylation of FAK and morphological changes such as rhomboidal changes, elongated changes, abnormal cytoplasmic protrusions, and membrane ruffling, in transfected cells. FAK inhibitor (1,2,4,5-Benzenetetraamine tetrahydrochloride) hindered the localization change of αIIbβ3 and the morphological changes in transfected cells by doxycycline induction. These results suggest that the gain-of-function mutation around membrane region of αIIbβ3 leads to macrothrombocytopenia with impaired surface αIIbβ3 expression.
antibodies against glycoprotein Ibβ (GPIibβ)IIa and/or GPIib/IIIa are considered to play a crucial role. B cell homeostasis and function are controlled by cell surface receptor-ligand interactions. The activation of PI3K is initiated by engagement of the pre-B cell receptor (BCR) and the BCR. The phosphatase and tensin homolog (PTEN) suppress the activity of the PI3K pathway. As a consequence, loss of PTEN function leads to excessive PI3, 4, 5 P3 at the plasma membrane and to recruitment and activation of Akt family members that potently drive cell survival and proliferation. PTEN regulates normal signaling through the B cell receptor (BCR). In immune thrombocytopenia (ITP), enhanced BCR signaling contributes to increased B cell activity, but the role of PTEN in human ITP has remained unclear. Both IL-21/IL-21R signaling and PI3K-PTEN molecules are involved in maintaining normal humoral immunity and deletion of autoreactive B cells. In this study, we want to determine whether abnormalities in PTEN might contribute to 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 patients.

Aims: PTEN is involved in maintaining normal B cell function. Since B cell overactivity is characteristic of immune thrombocytopenia(ITP) we sought to determine whether abnormalities in PTEN might contribute to increased B cell responsiveness in this disease.

Methods: 1. This study recruited 28 newly-diagnosed CITP patients and 26 sex and age matched health volunteers as health controls (HC). Peripheral blood mononuclear cells were isolated from collected anti-coagulated blood. 2. Flow cytometry and real time quantitative PCR were used for detecting the level of PTEN from PBMC cells of HC and CITP patients. 3. The relationship between PTEN levels and the disease severity of CITP was analyzed. 4. PBMC cells were incubated with human rIL-2 rIL-21 rCD40L or anti-IgM alone or in combination for 72h and after that the PTEN level was detected by flow cytometry. The proportion and surface activated marker of B cells were determined by flow cytometry.

Results: 1. Compared to HC the expression of PTEN was diminished in each CITP B cell population except IgD-CD38low/-memory B cells. In addition PTEN mRNA was also decreased in ITP B cells. 2. The level of PTEN in B cells was significantly lower in ITP patients (Figure 1). 3. The relationship between PTEN expression in B cells and the prognosis of ITP, which will provide a theoretical basis of new treatment strategy for the ITP patients.

Summary/Conclusions: Immune thrombocytopenia B cell showed decreased levels of PTEN and the decrease was associated with low platelet count and positive serum platelet-specific antibody. The capacity of IL-21 to induce PTEN was defect in CITP. Together, these data suggesting that the defective PTEN expression, regulation and function contribute to B cell hyper-responsiveness in CITP.
tin (PNA) that bind to galactose, N-acetyllactosamine and N-acetylgalactosamine residues, respectively. The NOD/SCID mouse model was used to study the impact of different glycan patterns on the survival of human PLTs.

**Results:** In this work 37 sera from ITP patients and 25 sera from healthy donors were analyzed. In the LBA, after incubation with AAbs, different patterns of glycan modification were observed. 17/37 sera caused a significant increase in PNA analysis compared to healthy donors (mean 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-IIIb/IIIa AAbs but also GPIb/IX 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% to 28%, range: 22-36%).

**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 THROMBOCYTOPENIA**

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

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

**Methods:** Whole exome sequencing (WES) was performed in 86 propositi with an unknown IT after the diagnostic workup based on the most updated diagnostic algorithm for ITs (Clin Genet 2016;89:141). RUNX1 variants detected by WES were confirmed by Sanger sequencing in the propositus and all available family members, which also underwent 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 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**

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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 <30x10^9/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 50-200x10^9/L. The primary endpoint was the % of time in the first 6 months with a plt response (plt count ≥50x10^9/L without rescue medication use in the past 4 weeks).

**Results:** As of 15 Mar 2016, 145 patients received ≥1 dose. At baseline, median (min-max) age was 10 (2-17) y; 51% were female; 4% had prior splenectomy. Median (min-max) ITP duration was 1.9 (0.5-12.3) y and plt count was 13 (2-168)×10^9/L. The median (Q1, Q3)% of time with a plt response in the
first 6 months was 50% (83.3%); that of months 7-12 was 92% (33%, 100%). Overall, 80% (114/143) of patients had a plate response. The median (Q1, Q3) of time with an increase in plt counts ≥20×10^9/L above baseline was 60% (25%, 84%). The median dose increased to 10 µg/kg by week 32. Median (min-max) treatment duration to date was 25 (1-67) weeks for a total exposure to date of 79 patient-years. Median (min-max) average weekly thrombolytic dose over the course of the study was 6.1 (0.4-9.0) µg/kg. 32 patients (22%) discontinued treatment for lack of efficacy (n=17), required other therapy (n=5), patient request (n=4), noncompliance (n=2), adverse event (AE) (n=2) (interstitial lung disease in a 15 y old boy and abdominal pain, vomiting, and headache related to treatment per investigator 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), petchiae (n=2), decreased plt count (n=2), and thrombocytopenia (n=2). A case of abdominal pain was the only SAE deemed treatment-related by the investigator. CTCAE grade 3 bleeding was seen in 8 patients (6%) and included epis-taxis (n=5), ecchymosis (n=2), petchiae (n=2), and 1 case each of hematemesis, hematemata, SC hemorrhage, injection site hemorrhage, and mouth hemorrhage. No grade 4 or 5 bleeding was observed. No neutralizing antibodies against romiplostim or TPO were identified. Of 30 patients with baseline bone marrow biopsies (bone marrow biopsies were obtained at European sites), all had modified Bauermeister scores of grade 0 (no reticulin) or 1 (fine fibers) and bone marrows typical for ITP. Of these 30 patients, 21 had evaluable on-study biopsies obtained after ~1 year of treatment, with no increases in 2 or more grades, findings of collagen, or bone marrow abnormalities (Figure 1).

Summary/Conclusions: In this first 1 year data of an ongoing open-label study of romiplostim in children with ITP, the% of time in the first 6 months with a plateau treatment response was 50%, with 80% of children having a plateau 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 with bone marrow biopsies. Future datacuts for years 2 and 3 in this study, the largest of romiplostim in children with ITP with 79 patient-years of exposure to date, will provide more information on plateau response, dose requirements, and safety.

Quality of life, palliative care, ethics and health economics 1

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PATIENT-REPORTED OUTCOMES AND HEALTHCARE RESOURCE UTILIZATION BEFORE AND DURING TREATMENT WITH ECULIZUMAB: RESULTS FROM THE INTERNATIONAL PAROXYSMAL NOCTURNAL HEMOGLOBINURIA REGISTRY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare disease caused by somatic phosphatidylinositol glycan class A (PIGA) gene mutation in bone marrow stem cells. Clinical manifestations may include fatigue, abdom-nal pain, dyspnea, dysphagia, erectile dysfunction, anemia, sudden hemoglobin level reductions due to complement-induced hemolysis, and PNH-related com-plications such as thrombosis, chronic kidney disease, and pulmonary hyper-tension, each of which impair quality of life (QoL) and could impact survival. Eculizumab, a humanized monoclonal antibody approved for treatment of PNH, reduces intravascular hemolysis, thrombosis rates, and other PNH-associated comorbidities. The International PNH Registry (NCT01374360) is an ongoing prospective, multinational, observational study established to record the natural history of patients with PNH and collect data on long-term efficacy and safety of eculizumab treatment.

Aims: Analyze patient-reported outcomes (PRO) and healthcare resource utili-zation (HRU) before and during eculizumab treatment.

Methods: Patient assessment questionaire (PAQ) data for patients with PNH who commenced eculizumab after Registry enrollment and had data available as of August 1, 2016, were analyzed. Patients had to have non-missing data on demographics, ≥1 recorded PAQ within 12 months prior to eculizumab ini-tiation, and ≥1 PAQ recorded ≥6 months after initiation. Outcomes of interest included changes in QoL assessments (Functional Assessment of Chronic Ill-ness Therapy (FACT)-Fatigue score; EORTC QLQ-C30 mean scores for global health/QoL, physical function, role functioning, emotional functioning, and social functioning), Karnofsky Performance scale, HRU, and missed work days.

Results: Of 4082 enrolled patients, 649 had non-missing data on demographics and initiated treatment with eculizumab as of August 1, 2016; 229 patients (55% female; 86% white; 74% from Europe) of the 649 met inclusion criteria for the current analysis. Median (min, max) interval between PNH disease start and initiation of treatment was 4.4 (0.1, 44.9) years. Clinically meaningful improvement in FACT-Fatigue score (≥4-point increase) was reported by 53% of patients after initiating eculizumab (mean change, 5.2 points. Figure 1). Clinically meaningful improvement (≥10-point increase) was also observed in EORTC QLQ-C30 mean scores for global health/QoL (mean change, 15.1), role functioning (16.3), emotional functioning (12.1), and social functioning (13.9) subscales. PNH-related symptoms disappeared in 19–44% of patients who reported the symptom prior to eculizumab across all assessed symptoms except erectile dysfunction, which did not disappear in any of the 21 patients who reported the symptom prior to eculizumab.

Conclusion: Eculizumab treatment was associated with clinically meaningful improvements in QoL assessments associated with PNH, Karnofsky Performance Scale, HRU, and missed work days.

Figure 1.
Summary/Conclusions: In this cohort of patients from the International PhN Registry, treatment with eczulizumab was associated with clinically meaningful improvements in PROs, including assessments of fatigue, global health status, patient functioning, and disease-related symptoms, as well as a decrease in emergency room visits and number of missed work days.

P370
ECONOMIC IMPACT OF INTRODUCING AGE-ADJUSTED D-DIMER CUT-OFF LEVELS IN THE DIAGNOSIS STRATEGY OF VENOUS THROMBOEMBOLISM
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Background: The diagnosis of venous thromboembolism (VTE) can be safely excluded in the case of D-dimer levels below a well defined cut-off value in patients with a low or intermediate pre-test probability (PTP), as the test negative predictive value (NPV) is close to 100%. As ageing is associated with increased D-dimer levels, the question arose whether D-dimer measurement was useful to rule out VTE in elderly patients.

Aims: The aim of the present study was to evaluate the clinical performance of a diagnosis strategy based on age-adjusted cut-off values calculated by multiplying the patient’s age by 10 in patients aged over 50, and to evaluate its economic impact.

Methods: We included 1255 consecutive outpatients with non-high PTP of VTE referred to the emergency departments at 5 French centres (2 university hospitals, and 3 general hospitals, in whom D-dimer testing was prescribed. The same standardized procedure was used in the 5 centres i.e. D-dimer measurement in patients with a non-high PTP, and imaging techniques (usually computed pulmonay angiography in case of suspected PE and Doppler ultrasonography in case of suspected DVT) in the case of D-dimer above the cut-off level. D-dimer levels were evaluated using the same fully automated latex-based assay (Hemosil®, Dade Behring, 100, Inspect and ren Laboratorium). The proposed cut-off level for VTE exclusion being 500 ng/ml (fibrinogen equivalent units, FEU).

Results: VTE diagnosis was established by objective testing in 115 patients (9.2%); 88 of the 1082 patients referred for suspected PE (8.1%) and 27 of the 173 patients referred for suspected DVT (15.6%). D-dimer levels were above 500 ng/ml in all patients with VTE and in 521 of the 1140 patients without VTE (45.7%), leading to test NPV and sensitivity of 100%. The overall test specificity was 54.3%, even though it significantly decreased in an age-dependent manner over 60 years old. This is due to increased D-dimer levels in older patients particularly in those above 80 years. Using age-adjusted cut-off levels, calculated by multiplying the patient’s age by 10, significantly improved the overall test specificity (60.2%). The NPV remained high (99.9%), even though a 78 years old female with a low PTP of PE would have been misdiagnosed as her D-dimer level (540 ng/ml) was above 500 ng/ml but below the age-adjusted cut-off value. Such an improvement in test performance was found both in patients with PE and DVT (Table). As such an increase in test specificity would have led to exclude VTE in a higher percentage of patients in the studied population, we evaluated the cost-effectiveness of both strategies, taking into account the local reimbursement rates of D-dimer testing, angiography and Doppler ultrasonography (18.20, 58.72, and 75.60 Euros respectively). The economic impact of the proposed diagnosis strategy was a decreased of 6.9% of total costs (45,023.4 48,356.4 Euros) for PE diagnosis and 5.1% (9,909 126,358.4 Euros) for DVT diagnosis vs 22,948, 25,300, and 28,660 Euros respectively, for PE diagnosis and 2,500, 4,000 and 5,500 Euros respectively, for DVT diagnosis.

Summary/Conclusions: The use of age-adjusted cut-off levels for D-dimer, in patients aged over 50 years old, led to a significant increase in the test specificity, but correlatively to slightly decreased NPV and sensitivity. Even though some patients with D-dimer levels above 500 ng/ml but below the age-adjusted cut-off could be misdiagnosed, such a strategy was found to be safe in our studied population with a high NPV (99.9%) and sensitivity (99.1%), and cost-effective.

P371
IMPACT OF CELLULAR THERAPY ON THE ECONOMIC BURDEN AND SURVIVAL FOLLOWING RELAPSE AFTER HLA IDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA AND MYELODYSPLASTIC SYNDROME
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Background: Relapse following allogeneic hematopoietic stem cell transplant (aHSCT) is associated to a very poor outcome and remains an unmet medical needs. The impact of treatment approach on costs and survival remains unknown. The development of innovative cellular therapy for the treatment of relapse following aHSCT may change its dismal outcome but the cost of such intervention has prohibited its large-scale development.

Aims: The objective of this study was to measure the economic burden associated with the management of relapse following aHSCT and to evaluate the impact of treatment choice on survival and health care costs.

Methods: A retrospective medical chart review was conducted at Maison- neuve-Rosemont Hospital (HMR) after research and ethic committee approval. Patients were selected using the Hematopoietic Stem Cell Transplant (HSCT) program database. Eligible patients were diagnosed with acute leukemia (AL) or MDS and relapsed following an aHSCT between January 1st 2011 and December 31st 2014. Patients’ and disease characteristics and relapse-related health care resource utilization were collected from the date of post transplant relapse until death or last follow-up. Canadian unit costs for each intervention/treatment were obtained from literature and governmental publications.

Results: During the study period, 645 HSCT were performed at HMR, 303 were allogeneic. A total of 36 patients met the inclusion criteria and were included in the analysis. 32 recipients were diagnosed with AL and 4 with MDS. Treatment approaches following aHSCT relapse were divided in three groups according to patient and physician choices: group 1 received supportive care (n=9), group 2 received chemotherapy or tyrosine kinase inhibitors (n=21) and group 3 received a cellular based therapy, either donor lymphocyte infusion (DLI) or a second aHSCT (n=6). The mean cost of care per patient per month was $20,239 (SD=17,079). The median survival following relapse for the entire cohort was 12.4 months (SD=2.8). For group 1, 2 and 3, the mean cost of care per patient per month was $17,436 (SD=16,447), $22,914 (SD=18,474) and $15,082 (SD=12,954), respectively. The median survival was 4.0 months (SD=2.0), 7.2 months (SD=1.6), and 46.6 months (SD=8.4), for treatment group 1, 2 and 3 respectively (Figure1).

Summary/Conclusions: Relapse following aHSCT is associated to a poor prognosis and survival and to significant use of health care resources. Despite the selection bias, only patients who received cellular based therapy, either DLI or another HSCT, enjoyed a prolonged survival. Healthcare resources devoted to the care of patients in relapse post aHSCT provide a comparative basis for cost efficiency analysis in the development of innovative cellular therapy.

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ACUTE MYELOID LEUKEMIA TREATMENT PRACTICE PATTERNS, HEALTHCARE RESOURCE UTILIZATION (HRU) AND COSTS IN A US COMMERCIALLY-INSURED POPULATION
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Background: AML is a rapidly progressive hematologic malignancy that accounts for 25% of all leukemias in the Western world, with estimated 5-year survival of 26%, and is associated with high HRU and costs.

Aims: To estimate HRU and costs among newly-diagnosed AML patients (pts) in a US commercially insured population by receipt of chemotherapy (CT) or stem cell transplant (SCT).

Methods: This was a retrospective observational study using the PharMetrics Plus® database. Pts were adults with AML (ICD-9-CM code 205.0x), 18 years and older, from a US commercially insured population. Pts with other malignancies, receipt of HSCT, or death within 3 months of AML diagnosis were excluded. AML diagnoses were identified between Jan 2007 and Jun 2016.

Results: Of the 5,316 newly diagnosed AML pts included in this study, 8% received CT only, 72% received CT plus SCT, 15% received SCT only, and 5% received supportive care only. Pts receiving CT plus SCT had significantly higher HRU and costs compared to CT only or SCT only. The median costs were $55,158 (IQR $18,691 – $118,238) and $54,902 (IQR $18,691 – $104,204), respectively, for pts receiving CT only or SCT only.

Summary/Conclusions: The impact of treatment approach on costs and survival remains unknown. The development of innovative cellular therapy for the treatment of relapse following aHSCT may change its dismal outcome but the cost of such intervention has prohibited its large-scale development.
not continuously enrolled for 12-months (mos) before the first AML claim (index date); evidence of acute promyelocytic leukemia anytime during the study period; missing enrollment information; or ≥1 hospitalizations during follow-up (FU) with missing cost. Pts were classified as treated or untreated, with treatment defined based on receipt of CT (inpatient or outpatient) or SCT. For treated pts, FU was partitioned into 2 periods: index date to 6 mos and >6 mos post index date. Mean HRU and costs over the FU period were calculated by receipt of treatment and, for treated pts, by time since index date.

Results: 10,197 pts met study criteria including 6,862 treated pts (67%) and 3,335 untreated pts (33%). Mean age was 55 and 60 years in treated and untreated pts, respectively. Mean follow-up was 19.3 mos in treated pts and 18.1 mos in untreated pts. Mean total costs were higher for treated pts ($386,711) vs untreated pts ($83,274). In treated pts, mean total costs were $166,156 during the first 6 mos (mean duration 3.9 mos), and $220,555 during the remaining follow-up period (mean duration 19 mos). 26% of treated pts had SCT. Costs of inpatient and outpatient CT during the first 6 mos were $86,188, representing 22% of the total cost for treated pts (Table 1).

Table 1.

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<th>Study Category</th>
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<th>Untreated Pts</th>
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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.

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HEALTH-RELATED QUALITY OF LIFE IN AL AMYLOIDOSIS PATIENTS WITH PERIPHERAL 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 sensorimotor 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-PN-affected (n=204) patients to assess patient characteristics and HRQoL (based on the SF-36v2® Health Survey [SF-36v2]). The survey measures eight health-related quality of life dimensions (physical functioning, role physical, role emotional, mental health, vitality, social functioning, general health, and health transition). Patients were then asked to select which of the following conditions they had: gastrointestinal involvement; musculoskeletal involvement, fatigue, and peripheral neuropathy. Statistical analyses included t-tests and linear models. Differences in mean HRQoL between AL-PN and non-AL-PN patients were compared to established minimally important differences (MIDs).

Results: Compared to non-PN-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-PN-affected patients. Nearly all AL-PN patients (97.6%) reported multi-system involvement. Gastrointestinal involvement was more prevalent in AL-PN patients versus non-AL-PN patients (68.3 vs 28.8%, p < 0.001). There were greater odds of experiencing numbness (OR=4.23, 95% CI: 2.45–7.30, p < 0.001) and fatigue (OR=3.09, 95% CI: 1.36–7.02, p < 0.01) among AL-PN patients as compared to non-AL-PN patients, even after controlling for other types of organ involvement. Similar findings were observed for gastrointestinal symptoms, such as alternating bouts of constipation or diarrhea (OR=1.92, 95% CI: 1.12–3.34, p=0.019) and early safety/feeling fullness in the stomach (OR=1.80, 95% CI: 1.03–3.16, p=0.04). With the exception of RE, MH, and MCS, there were significant differences in SF-36v2 scores among AL-PN patients as compared to non-AL-PN patients (p < 0.05 for all). These significant differences also exceeded the thresholds for clinically meaningful differences between the two groups.

Summary/Conclusions: This study suggests that the burden of illness from AL amyloidosis may be greater for those with PN involvement versus those without. AL-PN patients also experienced more complicated journeys to diagnosis and significantly worse symptoms related to nervous systems and physical 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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Background: Deciding how service delivery 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. Our target group was patients with myeloma, aiming for up to 20 a day once or twice a week.

Methods: The first drugs administered on the Mobile Unit were zoledroxate infusions, followed by bortezomib. When twice weekly doses were required, patients collected an additional injection pack which they could self-administer in their own homes, thereby saving another trip to hospital. Patients were given zoledroxate infusions, between taking 1–2 hours, were also administered. There was a consultant review clinic on board for patients receiving bortezomib which further reduced the numbers of hospital visits for patients and also a nurse-led Quality of Life assessment.

Results: In one year 548 treatments were administered on 91 days to a total of 54 individual patients. All 54 patients had a diagnosis of myeloma. 56% are female and 44% are male with an age range of 46 to 90 years of age, with 48% over 70 years of age. 37 patients are married and all but 4 classed themselves as White British. The greatest number of patients treated in a single day was 16. 98% felt safe having their treatment outside hospital and 92% said their experience was better than hospital. Patients could drive up to the door of the Mobile Unit and average time waiting from arrival to treatment chair was 2 minutes, with many people not having to wait at all. Uptake of the psychosocial support services was lower than expected with only 10 people opting for additional support. Any criticisms received focused on the locations we chose to site the Mobile Unit in relation to accessibility via public transport.

Summary/Conclusions: Treatment in the community alleviates the stress of treatment and with minimal waiting times it gives some patients the ability to maintain family life and where possible to continue to work. It is both feasible and acceptable to begin to ambulate many different sorts of treatments. The possibilities opening up for haematology include rituximab maintenance; community blood transfusions; delivering pentamidine for patients at risk of pneumocystis infection; late effects clinics for teenage and young adult cancer patients; and myeloproliferative neoplasm clinics, possibly near community pharmacies to facilitate dispensing medicines such as hydroxyurea.

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THE BUDGET IMPACT OF TREATMENT-FREE REMISSION FOR FIRST-LINE NILOTINIB OR GENERIC IMATINIB IN TREATING CHRONIC PHASE PHILADELPHIA-POSITIVE CHRONIC MYELOID LEUKEMIA
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Background: The 2013 European LeukemiaNet (ELN) guidelines recommend switching from first-line therapy as early as 6 months with poor molecular response (MR3.5-5). We also recognize the possibility of treatment-free remission (TFR), requiring deep MR (MR0 or MR4.5-5). These emerging shifts in practice will dramatically change chronic myeloid leukemia (CML) treatment patterns. Occurring in parallel to this is the introduction of generic imatinib in Europe,
which will have a substantial price difference compared with nilotinib. However, given the possible changes in switching and TFR in this model, this price difference may not translate into a similar magnitude of difference in drug budget for first-line nilotinib vs imatinib due to better MR with nilotinib.

Aims: To estimate the budget impact for first-line nilotinib vs imatinib when considering generic imatinib pricing, early treatment-switching, and TFR. Methods: The model was based on the Patient Regimen Simulator (PRIS) and 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 assumption was patients could enter first-line or second-line TFR after 36 months of continuous therapy where the last 12 months were at MR4.5.

Duration of first-line or second-line TFR was based on an extrapolation of ENESTnd. A greater number of patients in the first-line nilotinib arm remained on first-line therapy (690 vs 479 at 15 mos., and 542 vs 366 at 60 mos.); achieved a complete cytogenetic response (CCR) (442 vs 248 by 60 mos.); entered TFR 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 that would be expected to occur in DEFACTO. If the model 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 switchout benefit of a lower imatinib acquisition price. The median period between first-line imatinib and nilotinib would be further reduced by TFR, which occurred in the model more frequently in the nilotinib group. The superior efficacy of nilotinib and the associated differences in switching and TFR eligibility are predicted to substantially offset the lower unit cost for generic imatinib.

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GAH SCALE PREDICTS TREATMENT TOLERABILITY IN OLDER PATIENTS (>65 YEARS) DIAGNOSED WITH HEMATOLOGICAL MALIGNANCIES
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Background: The Geriatric Assessment in Hematology (GAH) scale is a newly developed tool that is intended to be an ancillary questionnaire to better categorize elderly patients diagnosed with hematologic neoplasms and is intended 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 were initially dichotomized into 0 or 1. The GAH scale has recently been shown to be psychometrically valid, responsive to clinical change, and able to predict survival.

Aims: To determine the weights for each dimension of the GAH scale and the cut-off points for the scale to be used as a tool to predict treatment tolerability in older patients diagnosed with myelodysplastic syndrome / acute myeloblastic leukemia, multiple myeloma, or chronic lymphocytic leukemia. Methods: A prospective, observational study conducted at 14 Spanish sites. Prior participants of the GAH study were given treatment within 3 months after having completed the GAH scale were eligible for inclusion after giving informed consent. A logistic regression model and a full multiple linear regression model were calculated to determine the weights for each dimension and finalize its contribution to the final score; the ROC curve analysis was used to calculate the cut-off points that defined three groups: “go-on” (low probability to develop toxicity regardless of intensive or attenuated therapy), “slow-go” (high probability to develop toxicity with intensive therapy but low probability with attenuated therapy), and “no-go” (high probability to develop toxicity regardless of therapy). 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, and 60 for activities of daily living, 6 for subjective health status, 27 for nutrition, -5 for mental status, and 1 for comorbidities. The sum of the GAH scale score, ranging from 0 to 66, plus a factor derived from the treatment intensity (34 points for intensive therapy or 0 for attenuated) leads to a maximum score of 100 points, at which a cut-off point set at 47. Figure 1 shows the GAH scale equation for treatment toxicity prediction and the classification of patients according to their score. Among the 97 (89.8%) patients that received intensive therapy, 26 patients were classified as go-on, 48 as slow-go, and 23 as no-go. The proportion of patients that developed toxicities for each group was 34.6%, 56.3%, and 78.3%, respectively, with a statistically significant difference (P=0.002).

Figure 1.

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

P377
NUTRITIONAL NEEDS AND PREFERENCES OF MYELOPROLIFERATIVE NEOPLASM PATIENTS: PHASE IA OF THE NUTRIENT STUDY
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Background: Cachexia, weight loss, and malnutrition in cancer patients are important contributors of adverse outcomes of cancer patients. MPN patients have abnormal cytokine expression (e.g., IL-1, IL-6, IL-8, and TNF-a) that contributes to symptom burden (e.g., fatigue, pruritus, night sweats, bone pain) and decreased functional status (e.g., reduced physical activity). The most common adverse symptoms are fatigue, loss of appetite, and low-fat dairy products are associated with a decrease in inflammatory (e.g., TNF-a, IL-8, and CRP) and thrombotic markers (e.g., homocysteine, fibrinogen). Chrysohoou 2004, Smidowicz 2015). To date, no studies have evaluated the nutritional needs or preferences of MPN patients in regards to dietary treatment.

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 Research 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 MPN-SAF TSS/MPN-10 (Emanuel 2012).

Results: Demographics and symptom burden: 919 international MPN patients took the survey online, and the online survey was distributed to 22,5% were diagnosed with MF, 37.1% with PV, and 37.4% with ET. Respondents represented MPN patients from 37 States (48.8%), United Kingdom (32.7%), Australia (6%), and Canada (3.6%). Average MPN-SAF TSS score was 33.6 (SD=17). Dietary Habits: 22.5% of the respondents having dairy products or high-fat foods (e.g., milk, cheese, or salad dressing). Specifically, 31.7% of individuals followed a specific diet or had dietary restrictions. On average, MPN patients ate 2.1 meals outside the home per week. Among those taking supplements, 16.6% took supplements with the intent of reducing inflammation. Half (47.5%) of these individuals felt that the supplements they used made them feel better. Approximately 15% of respondents had tried alternative medicine to help treat their MPN. Among these, 44.8% were under the care of a naturopath and 60.2% endorsed that their treatment plan included dietary change. Nutritional Education Preferences: Overall, 34.4% of patients endorsed
using diet to help control their symptoms or MPN disease. Patients most often utilized books (28.2%), websites (27.1%), health care providers such as physicians, NPs or naturopaths (28.2%), online forums (23.2%), friends (12.2%), nutritionists (9.5%), phone or tablet applications (9.1%), or videos (4.2%) for nutritional education. The vast majority (95.9%) of MPN patients endorsed being willing to eat only certain foods if it helped to control symptom burden and or could help their MPN to stabilize or reduce the risk of their MPN getting worse (98.0%).

**Table 1.**

<table>
<thead>
<tr>
<th>Frequency of dietary allergens, intolerances, restrictions and supplementation use across a large national cohort of MPN patients (n=419).</th>
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<tr>
<td><strong>Food group</strong></td>
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<td>Milk</td>
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<td>Wheat</td>
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<td>Fruit</td>
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<td>Meat</td>
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Summary/Conclusions: There remains an unmet need for symptom burden improvement in low-risk MPN patients or among those who have reoccurrence of symptoms while on JAK inhibitor therapy. Nutritional interventions for MPN patients have not previously been investigated and have the potential to be paired with traditional interventions to allow MPN patients to self-manage symptom burden. This study represents the first evaluation of MPN-related nutritional habits and preferences. These results will be used to inform the creation of an MPN nutritional intervention with the goal of improving symptom burden and reducing inflammation.

**P378**

**DO PHYSICIANS NEED HELP TO ADEQUATELY INFORM AND SUPPORT PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA? RESULTS FROM A QUALITATIVE STUDY IN GREECE**

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Background: Despite recent progress in prognostication and management, chronic lymphocytic leukemia (CLL) remains unpredictable at diagnosis, while virtually incurable, posing challenges to physicians on how to properly communicate the actual nature of the disease. Moreover, the great majority (~85%) of patients do not need treatment at diagnosis, creating a major cognitive dissonance between the perception of leukemia diagnosis and the “wait & watch” strategy usually applied, that may become a major reason of anxiety and quality of life (QoL) impairment for patients and frustration for physicians. Evidently, both patients and physicians need parameters that would allow co-decision making tailored to each particular case.

Aims: To identify physicians’ needs in order to improve their communication skills and thus facilitate CLL patient empowerment through a patient-centered-ness model.

Methods: An in-depth qualitative study with semi-structured interviews was conducted within hematologists (n=30) all over Greece. Data collection was considered as completed when saturation was reached i.e. no new themes emerged as assessed by the investigators. Content analysis was performed separately by a hematologist and a health psychologist with 98% inter-rater reliability score.

Results: None of the participants had ever received formal communication training but rather adopted the techniques of senior physicians or developed their own through experience alone, thus frequently doubting their approaches (n=12/30, 40%). The most popular communication technique mentioned was adaptation of the quality and quantity of information provided according to each patient’s characteristics (n=29/30, 96.7%); followed by the use of caregivers as mediators for the communication of difficult issues (n=24/30, 80%); balance of realism and hope (n=21/30, 70%); careful choice of wording (e.g. lymphocytosis instead of leukemia) (n=18/30, 60%); gradual disclosure (n=17/30, 56.7%); and, descriptions through pictorial representations or metaphors (n=16/30, 53.3%).

Even though physicians did not systematically assess patients’ anxiety and depression levels, they often found themselves dealing with patients’ emotions (n=29/30, 96.7%) through lengthy discussions. With regards to decision making, some mentioned that physicians should make all the decisions (n=9/30, 30%) and that patients are not always willing to take part in the decision-making process (n=8/30, 26.7%), while others were keener on stirring patients towards a decision (n=15/30, 50%), taking into account patients’ preferences (n=10/30, 33.3%). Most physicians felt uncomfortable delivering bad news such as initial diagnosis, relapse and poor prognosis (n=25/30, 83.3%). Self-reported needs included (i) communication skills training (n=20/30, 66.7%); (ii) psychological support (n=7/30, 23.3%); and, (iii) working in a multidisciplinary team (n=8/30, 26.7%).

Summary/Conclusions: In the absence of structured communication guidance there is great uncertainty among physicians concerning their skills on communicating CLL nature and handling difficult situations, leading to distress endangering their engagement in a healthy relationship with the patient. Additional studies are warranted at European level for identifying physician needs in different countries aiming at improving their communication skills to support and empower CLL patients for participating in their own care and enhance their QoL.
Methods: CR using digital droplet (dd) PCR.

Aims: MN1 pts (ASH 2016, #517). Until today no study assessed the prognostic impact of relapse (CIR) for pts with high pre-HSCT outcomes. At diagnosis high expression of the AML associated genes disease detection. Recently, we demonstrated a higher cumulative incidence allogeneic stem cell transplantation (HSCT) have a dismal prognosis. Identifi-

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 patients between 50-70 years who were performed for the whole group and separately according to disease status at SCT (CR1, CR2, above).

Methods: AML patients aged between 50 and 90 years old receiving a first or second allo SCT between 2004 and 2014 with MSD or UDT 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 II/III, IV, 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 32-37) vs 37% (95%CI 34-40) in pts above 70 and 24% (25% vs 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 35% (95% CI 32-38) vs 45% (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 27-47) 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.
Sickle Cell Disease (SCD) and Diamond-Blackfan Anemia (DBA). Bertain 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/III trial evaluates the safety and efficacy of post-transplant infusion of donor T-cells transduced with the iCD9 suicide gene (BPX-501 cells). (ClinicalTrials.gov identifier: NCT02055869). The iCD9 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 o/- 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, thiopepa 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 observed at a median of 23.5 days (range 14-55) and there were two patients re-hospitalized at 30, 163 days respectively. Grade III/IV skin acute GVHD occurred in four patients and one patient had acute skin GVHD Grade IV. No chronic GVHD was observed. The 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). The median time to last RBC transfusion was 8 days (5-34 days). See Figure 1 for individual Hemoglobin levels. Median time of infusion of 1×10^6 BPX-501 T cells/kg was 14 days after HSCT (range 10-26). BPX-501 cells expanded after infusion and still persist in all patients. Immune reconstitution was normal cellular and humoral immunity present at 168 days post HSCT. All patients remain transfusion-free with a median hemoglobin of 11 or greater after 6 months.

Figure 1.

Summary/Conclusions: These data suggest that Haplo-HSCT combined with infusion of BPX-501 T cells with a suicide gene may be a safe and curative option for children with hemoglobinopathies and ED who lack a matched donor. Infusion of gene modified T cells with an inducible suicide mechanism, combined with selective o - 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.

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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 POSILIZED BM-SCDS OF MULTIPLE DONORS. P. Bader1, Z. Kuc1, S. Bakhtrian1, O. Basu2, G. Bug3, M. Dennis4, J. Greil5, K. Källay6, P. Lang7, G. Lucchini8, R. Po9, A. Schulz10, K.-W. Sykora11, I. von Lueettichau12, P. Remény13, L. Gopcsa13, A. Jarisch1, J. Soerensen1, K. Kállay6, P. Lang7, G. Lucchini8, R. Pol9, A. Schulz10, K.-W. Sykora11, E. Salzmann11, E. Seifried14, T. Klingebiel1, H. Boenig14, S. Kuci1,

Background: All clinical data published thus far on the use of MSCs were generated using cells expanded from individual bone marrow donor 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-phase-1 vials from which end-of-phase-2 MSC products are expanded for clinical use. The manufacturing process is fully GMP-compliant and generates an animal serum-free product with near-identical phenotype and in-vitro immunomodulatory potency. Importantly, they showed a significantly higher allo-suppressive potential than the mean allo-suppressive potential of MSCs generated from individual donors. All tested individual MSC doses were equipotent in suppression of the alloantigen-specific reaction in mixed lymphocyte reactions (Kuc1 et al. Haematologica 2016: 101 (8): 885-894).

Aims: A “hospital exemption” issued by the national regulatory authority Pau-Ehrlich-Institute (Number: PEF: A11748.0.1) 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 standard standard steroid treatment and treatment with non-malignant diseases. Median age was 8 years (range: 0.5-57 years). The stem cell sources were BM (n=17, 13%), SE (n=33, 25%) or MMFD (n=19, and derived from BM (n=27, 52%), peripheral blood (n=24, 46%) or cord blood (n=1, 2%). Patients were suffering from aGVHD grade II (n=3, 5.5%), III (n=14, 27%), or IV (n=31, 60%) or extensive cGVHD (n=7, 4.5%). Acute GVHD occurred at a median of 52 days (5-200 days) after transplant. Patients received in weekly intervals up to four MSC infusions after having failed to respond to the treatment with either two lines (n=10, 19%), three lines (n=20, 38%), four lines (n=10, 19%), 5 lines (n=7, 13%), six lines (n=4, 8%), or 7 lines (n=1, 2%) of immune suppressive drugs.

Results: Response was defined as either complete response (CR) in patients who showed one overall GVHD grade less according to the Glucksberg criteria, or non response (NR) at day 28 after first MSC transfusion. At day +28, 12 patients (23%) achieved CR, 29 patients (57%) PR (overall response( 80%), 8 patients (17%) NR, and in 2 patients (4%) no data were available at day +28. At the last follow up of GVHD, 29 patients (56%) were in CR, 13 patients (25%) in PR, 9 patients (17%) in NR, and for 1 patient (2%) no data were available. At 2 years these response rates resulted in a non-relapse mortality rate (NRM) of 27±6%, cumulative relapse incidence (CIR) of 14±5%, and a 2-year overall survival rate (OS) of 52±9%. Patients with aGVHD III and IV had an OS survival probability at 2 years of 77±12% and 59±35%, respectively thus dramatically in excess of expected survival rates for patients with such severe aGvHD. There was no difference between younger (n=40) and older patients (n=12) than 16 years.

Summary/Conclusions: Treatment with standardized equipotent MSCs from the "FRANKFURT MSC-BANK" offers an excellent chance to overcome treatment-resistant and steroid-refractory acute GVHD.

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HIGHER PEAK TACROLIMUS CONCENTRATIONS AFTER ALLOGENEIC TRANSPLANTATION INCREASE THE RISK OF ENDOTHELIAL CELL DAMAGE AND COMPLICATIONS. T. Morishita1,*, M. Ohbiki1, M. Osaki1, M. Yoshino1, S. Ikeno1, M. Nakashima1, T. Sato1, Y. Kagaya1, Y. Ozawa1, K. Miyamura1, K. Miyamura1, I. Hematology, Japanese Red Cross Nagoya Daiichi Hospital, Nagoya, Japan.

Background: Noninfectious transplantation-related complications (TRC) such as GVHD and endothelial cell damage (TRC-EC) including sinusoidal obstructive syndrome (SOS), transplant-associated microangiopathy (TAM), idiopathic pneumonia syndrome (IPS) are dismal complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Calcineurin inhibitors such as tacrolimus (TAC) have been broadly used to manage post-transplant complications. Higher blood levels of TAC are expected to reduce the risk of GVHD, but may increase the risk of endothelial damage. Since TRC-EC often developed in patients with severe GVHD, it is difficult to judge appropriateness of immunosuppression clinically.

Aims: Here we evaluated the impact of TAC blood levels upon TRC-EC occurrence and prognosis after allo-HSCT.

Methods: Two hundred sixty-one consecutive patients (pts) who received TAC as a GVHD prophylaxis after allo-HSCT at our institute from 2009 to 2015 were candidates for this retrospective study. Pts who received haploidentical allo-HSCT haematologica | 2017; 102(s2) | 131
and pts with unavailable TAC concentration data were excluded. A total of 253 pts was eligible. All pts received standard GVHD prophylaxis by continuous intravenous (iv) TAC with starting dose of 0.02 mg/kg/day from 1 day before allo-HSCT (day -1) and iv methotrexate on day 1, 3, 6 at dose of 10 mg/m², 7mg/m², respectively. TAC dosage was adjusted to target the serum concentration of 8-12 ng/ml until at least day 30 and then tapered. TAC was rapidly tapered in case of the pathological diagnosis of TAM. TAC serum concentration was sequentially examined tri weekly until day 35 at least. The primary endpoint of this study was to evaluate the cumulative incidence of TRC-EC in relation to weekly mean/peak TAC concentration. Secondary endpoint was OS.

Results: Median patient age was 45 years (16-68). The risks of disease were standard in 168 and high in 85 pts. Forty pts were diagnosed of TRC-EC: S0S: 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=0.018) and pts with unavailable TAC concentration data were excluded. A total of 253 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 transplanted for de novo AML, p=0.01. No differences were found on disease status and Karnofsky performance status (KPS) at transplant: pts were in CR1 (MAC: 44%, RIC: 40.5%), CR2/3 (MAC: 17%; RIC: 17%) or had active disease (MAC: 40%; RIC: 43%), p=0.68; 12% of pts in both groups had KPS>80, p=0.95. The most frequently used MAC regimen was TBF (56%), while in RIC it was miniTBF (27%) and low dose TBI+Fludarabine (24%). RIC regimens had a more frequent conditioning regimen associated with stem cell source (MAC 42% vs RIC 55%, p=0.002). Post-transplant cyclophosphamide was used in 69% of both RIC and MAC, p=0.39. Main outcomes were not different according to conditioning regimen: at 2 years RIC was 36% 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% (p=0.94), LFS 42% 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 followed by disease recurrence. In multivariate analysis, the type of conditioning regimen was not associated with risk of relapse or treatment failure: RI (HR: 1.22, p=0.28), NRM (HR: 0.92, p=0.63), acute GVHD grade II-IV (HR: 1.14, p=0.48), chronic GVHD (HR: 1.26, p=0.30), LFS (HR: 1.03, p=0.77), GRFS (HR: 1.07, p=0.55), OS (HR: 1.05, p=0.68). Disease status was associated with outcomes (active disease vs CR): RI (HR: 2.44, p<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 in conditioned patients. 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 comparing RIC vs MAC for haplo-SCT in adult pts with AML.

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

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Background: Patients with aggressive adult T cell leukemia-lymphoma (ATL) are candidates for upfront 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.

Summary/Conclusions: This study was planned to compare the outcome of RIC and MAC in pts with AML older than 45 years (yrs) undergoing haplo-SCT. The aim of the study was to confirm the efficacy and feasibility of RIC among a population for which the choice of conditioning intensity is more 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 transplanted for de novo AML, p=0.01. No differences were found on disease status and Karnofsky performance status (KPS) at transplant: pts were in CR1 (MAC: 44%, RIC: 40.5%), CR2/3 (MAC: 17%; RIC: 17%) or had active disease (MAC: 40%; RIC: 43%), p=0.68; 12% of pts in both groups had KPS>80, p=0.95. The most frequently used MAC regimen was TBF (56%), while in RIC it was miniTBF (27%) and low dose TBI+Fludarabine (24%). RIC regimens had a more frequent conditioning regimen associated with stem cell source (MAC 42% vs RIC 55%, p=0.002). Post-transplant cyclophosphamide was used in 69% of both RIC and MAC, p=0.39. Main outcomes were not different according to conditioning regimen: at 2 years RIC was 36% 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% (p=0.94), LFS 42% 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 followed by disease recurrence. In multivariate analysis, the type of conditioning regimen was not associated with risk of relapse or treatment failure: RI (HR: 1.22, p=0.28), NRM (HR: 0.92, p=0.63), acute GVHD grade II-IV (HR: 1.14, p=0.48), chronic GVHD (HR: 1.26, p=0.30), LFS (HR: 1.03, p=0.77), GRFS (HR: 1.07, p=0.55), OS (HR: 1.05, p=0.68). Disease status was associated with outcomes (active disease vs CR): RI (HR: 2.44, p<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 in conditioned patients. 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 comparing RIC vs MAC for haplo-SCT in adult pts with AML.
Decision analysis is a computerized modeling analysis which can simulate the clinical outcomes of different therapeutic strategies and identify an appropriate therapeutic strategy.

**Aims:** The aim of this study is to compare the life expectancy (LE) of chemotherapy followed by up-front allo-HSCT to that of chemotherapy alone using decision analysis in patients with aggressive ATL using database constructed by a nationwide survey.

**Methods:** We constructed a Markov decision analysis model to compare the outcomes in 2 therapeutic strategies: chemotherapy followed by up-front allo-HSCT vs chemotherapy alone. The transition probabilities between each health states were calculated from the database of 1,792 patients and patients were stratified into low-, intermediate- and high-risk groups according to the risk stratification system which we developed previously (Fuji S et al. 18th International Conference on Human Retrovirology). The model simulated the LE, quality-adjusted LE (QALE) and survival curve after diagnosis of aggressive ATL. Since QoL data for patients with aggressive ATL are lacking, estimated QoL was used in a similar decision analysis study of patients with acute myeloid leukemia were used. In terms of the timing of up-front allo-HSCT, it was set as all patients receive up-front allo-HSCT from 2 to 6 months if ATL did not progress before allo-HSCT. We used the TreeAge Pro 2016 software package for decision analysis (TreeAge Software Inc., Williamstown, MA).

**Results:** In all patients, up-front allo-HSCT was associated with higher LE in comparison to chemotherapy alone (2.26 years vs 1.75 years). Stratified into 4 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 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 TRANSPLANT

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**Background:** Cord blood transplantation (CBT) is an established alternative source for hematopoietic stem-cells in patients without matched donor. However, the most commonly used high-dose total-body-irradiation (TBI) myeloablative conditioning (MAC) results in high treatment related mortality (TRM). Non-myeloablative and reduced-intensity conditioning (RIC) have been studied to decrease TRM and provide curative chance to the elderly and those with comorbidities. However, these strategies are associated with higher relapse-rate and graft rejection. A novel-RIC using addition of thiopeta and higher dose of TBI to standard RIC has shown to result in sustained donor engraftment. 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 (200cGy or 300cGy) versus this standard-RIC regimen with addition of thiopeta (10mg/kg) and increased dose of TBI (6-8cGy).

**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 thiopeta 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:** Of the 99 patients who underwent allogeneic double-CBT, 52 received standard-RIC and 47 received novel-RIC. Median age at transplant was 67 years (range, 24-74) and 54 years (range, 25-67) in standard-RIC and novel-RIC cohort respectively. Acute myeloid leukemia was the major indication for transplant in both cohorts. Median hematopoietic stem-cell transplant comorbidity-index (HSCT-CI) was 3 (range, 0-6) and 1 (range, 0-4) 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%) patient (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 not significantly improved in novel-RIC cohort compared to standard-RIC (HR, 0.32, CI:0.11- 0.76, p=0.01). Median RFS was 29 months in standard-RIC cohort while median RFS was not reached in novel-RIC cohort. The one-year cumulative incidence of transplant related mortality (TRM) was 22% (n=10) in those who received standard-RIC while it was 16% (n=7) in those who received novel-RIC. TRM was not significantly different between the standard-RIC and novel-RIC cohorts. Median follow-up in standard-RCI cohort was 9.3 months (range, 0.16- 79) and 13 months (range, 1.4- 36) in novel-RCI cohort. The overall survival (OS) was significantly better in novel-RCI cohort compared to standard-RCI (HR 0.49, CI:0.25- 0.94, p= 0.03). Median OS was 17 months in standard-RIC cohort while median OS was not reached in novel-RIC group (Figure 1).

**Summary/Conclusions:** In our study, RIC consisting of FluCy with addition of thiopeta and increased dose of TBI in patients undergoing double-cord UCT was associated with improved OS and improved RFS without increase in TRM compared to standard RIC. While older and more comorbid patients might experience increased TRM with the thiopeta 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 stem-cell transplantation (allo-HCT), MRD-directed intervention may be a reasonable option for relapse prophylaxis.

**Aims:** We investigated the efficacy of MRD-directed interferon-α (IFN-α) treatment in acute leukemia patients who were positive for MRD after allo-HSCT.
Methods: A total of 102 patients who were MRD-positive after allo-HSCT were enrolled. MRD-positive state was defined as positivity for leukemia-associated aberrant immune phenotypes or positivity for Wilms' tumor gene 1 in a single bone marrow sample. Recombinant human IFN-α-2b injections were administered subcutaneously 2–3 times per week for 6 months.

Results: The 2-year cumulative incidence of severe acute and chronic graft-versus-host disease (cGVHD) with AZA or BSC was 56% (95% CI: 48–64) and 66% (95% CI: 58–74), respectively. Eighty-one (75.7%) patients treated MRD-negative after IFN-α treatment, including 42 (39.3%), 6 (6.6%), 7 (6.5%), and 26 (24.3%) who treated MRD-negative 1, 2, 3, and >3 months after MRD-directed IFN-α treatment, respectively. Twelve patients showed relapse 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 32.3%, 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 MYELOIDYSPLASTIC 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 available for MDS. In recent years, azacitidine (AZA) has been increasingly used as pretransplant induction therapy in high-risk MDS patients. However, the benefits of pretransplant therapy in these patients are unclear, and the optimal therapy regimen remains unknown.

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

Methods: Clinical data were collected from the registry database of the Japan Society for Hematopoietic Cell Transplantation. We selected patients with high-risk MDS at diagnosis (IPSS intermediate 2 or high), aged 16 years or older, who underwent their first transplantation between January 2009 and December 2014 and received AZA or BSC as best supportive care (BSC) before allo-HSCT. Patients who received conventional chemotherapy or immunosuppressive therapy prior to allo-HSCT were excluded. We compared overall survival (OS), relapse, non-relapse mortality (NRM), and hematopoietic recovery after allo-HSCT between patients who received AZA or BSC as pretransplant 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.

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LOW-DOSE DECITABINE IMPROVES PLATELET RECOVERY IN PATIENTS WITH ISOLATED THROMBOCYTOPENIA AFTER HSCT

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

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

Methods: We performed a prospective open-label study to evaluate the treatment of low-dose decitabine in patients with hematological malignancies who received allogeneic HSCT and suffered from isolated thrombocytopenia. The inclusion criteria were: (1) Platelet count ≤ 100 × 10⁹/L persistently at day 60 post-HSCT or later; (2) Recovered neutrophil and hemoglobin; (3) Full donor chimerism; and (4) No response to conventional treatments for a duration of at least 4 weeks. Patients with malignancy relapse, active infections, uncontrolled graft-versus-host disease, severe organ damage or transplant-related thrombosis were excluded. From July 2013 to July 2016, 38 patients were randomly assigned into either the control group to receive conventional treatment only, or the test group to receive additional decitabine (15mg/m², intravenously daily for the first 5 days). Results: Major response was observed in 16 out of 19 patients (84.2%) in the decitabine group, with a median time of 22 days to achieve platelet transfusion-independence. Two patients (10.5%) showed a minor response and 1 patient (5.3%) failed. In contrast, 3 out of 19 patients in the control group (15.8%) showed a major response, 2 patients (10.5%) showed a minor response, 14 patients (73.7%) did not show any improvement, of which 1 patient died of severe hemorrhage in week 5. For bone marrow morphological analysis, all 38 patients showed low levels of megakaryocytes at week 0. However, the megakaryocyte counts in decitabine group were significantly increased at week 4, while no significant difference was recorded in control group. After decitabine treatment, we did not observe a change in anti-platelet antibodies levels and T cell subsets ratios. However, reactive oxygen species (ROS) and megakaryocyte counts increased in the test group. No considerable myelosuppression, febrile neutropenia, and nonhematologic toxicities associated with the treatment were observed.

Summary/Conclusions: Our data showed an encouraging efficacy of decitabine in patients after HSCT suffering from isolated thrombocytopenia, and it could remarkably increase megakaryocyte counts. Decitabine may improve isolated thrombocytopenia via regulating ROS and megakaryocyte reconstitution.

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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 these genetic 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 1,272 proteins. Many proteins were previously reported EV constituents. 19 proteins were consistently increased in patient samples compared to controls across all data sets. The majority of these proteins were chaperone proteins and antioxidant enzymes. Alpha Hemoglobin Stabilizing Protein (AHSP) had the highest extent 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.

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A SELECTIVE ORAL GLYT1 INHIBITOR IMPROVES ANEMIA IN A MOUSE MODEL OF BETA-THALASSEMIA

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Background: The anemia of β-thalassemia is due to a combination of reduced red cell survival in the peripheral circulation and ineffective erythropoiesis, the latter due to unbalanced hemoglobin chain synthesis, and hemichrome-induced oxidative damage. Here we used a specific and selective inhibitor of the plasma membrane expressed glycine transporter GlyT1 selective inhibitor RO4917838 (GlyT1) in a mouse model for β-thalassaemia (Hbb3th/+). A previous study in Wistar rats has shown that RO4917838 administration was associated with an improvement of β-Thal hematologic phenotype, as supported by (i) the amelioration of red cell morphology; (ii) the increase in Hb levels; (iii) the reduction in reticulocyte count.

Aims: To evaluate the impact of the glycine transporter GlyT1 selective inhibitor RO4917838 on anemia of a mouse model for β-thalassemia.

Methods: Wild-type control (WT) C57B/6J and Hbb3th/+ mice (β-Thal) aged between 3 to 4 months were treated daily with either vehicle or RO4917838 at dosages of 3, 10, 30 mg/kg/d for 4-6 weeks by gavage. Hematological parameters, analysis of erythropoiesis, molecular studies of sorted erythroid precursors, indices of hemolysis, hepcidin liver expression and Pearsall staining were carried out.

Results: RO4917838 administration was associated with an improvement of β-Thal hematologic phenotype, as supported by (i) the amelioration of red cell morphology; (ii) the increase in Hb levels; (iii) the reduction in reticulocyte count.
and in the percentage of circulating erythroblasts; (iv) the increase in β Thal red cell survival. RO4917383 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, inducing a reduction in free heme, which shall resulted in the activation of HRI, in RO4917383 treated β -Thal mice (10 mg/kg/d, 6 weeks). Finally, in β-Thal mice treated with RO4917383 (4 weeks at 30 mg/kg/d) a reduction in liver and spleen iron-overload was identified, which was associated with increased hepcidin liver expression.

Summary/Conclusions: Our data suggest that RO4917383 ameliorates anemia and ineffective erythropoiesis by reduction of heme biosynthesis in a mouse model for β-thalassemia. RO4917383 is a potential, novel therapeutic approach for the treatment of anemia in patients affected by beta-thalassemia.

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MAY MUTATIONS IN THE KLF1 GENE HAVE WORSENING EFFECTS ON THE BETA THALASSEMIA PHENOTYPEN

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Background: KLF1 is a pleiotropic erythroid transcriptional factor that plays a key role in erythropoiesis (Siatecka M, Blood 2011; 118: 2044-521). Accordingly, KLF1 mutations have been found to be responsible for a variety of hematological disorders. KLF1 also contributes directly or indirectly to regulate the expression of genes in the beta-globin gene cluster and the fetal-to-adult globin gene switch (Wayne JS et al Int. J. Lab. Hem. 2015; 37: 78-84). It has been reported that mutations leading to KLF1 haplosufficiency (i.e. a dominant negative KLF1) can result in hematologic and/or metabolic abnormalities (Smitz et al, Blood 2008; 111: 614-620) and ameliorate the severity of β-thalassemia (Liu D. et al, Blood 2014; 124: 803-811; Perkins A. et al, Blood 2016; 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 (moderate or severe anemia, elevated HbA2 and/or HbF levels) and 8 subjects with normal erythrocyte indices and borderline HbA2 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 in the transcribed region of the first exon (S102F, F182L and M39L), (Radmilovic M. et al, Ann. Hematol 2013; 92: 53-58) and 2 novel mutations (C94X and P173PfsX236), all of them in the proline-rich domain in exon 2. Functional studies were performed in K562 cells in order to clarify the pathogenic significance of these mutations and to better define the role of KLF1 in atypical thalassemia phenotypes. Interestingly, the c.-251 C>G polymorphism was found to be associated with an increased transcriptional activity of the KLF1 promoter (Figure 1A), thus allowing us to exclude for this nucleotide variation the condition of a neutral polymorphism. Furthermore, unexpectedly, the novel P173PfsX236 mutation was found to be associated with a dramatic reduction of the beta-globin gene expression (Figure 1B).

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

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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 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 time of this report, 112 patients were cured, whereas 5 patients rejected their graft and are now under regular transfusion treatment. Overall, the median follow-up after HCT was 24 years (3-34). Seven patients developed malignancy 6 years (median age at time of malignancy 10 years after HCT including 2 carcinomas of the tongue, 1 thyroid carcinoma, 1 carcinoma of the uterine cervix, and 1 parotid carcinoma. The 30-yr cumulative incidence (CI) of developing SST was 10±0.17%. All patients underwent surgical resection of the tumor and in addition of 4 of them received chemotherapy and/or radiotherapy. Of relevance, the 3 patients with cancer of the oral cavity were affected by severe chronic GVHD with buccal cavity involvement. Two patients (1 with parotid and 1 with tongue carcinoma) died of tumor progression and 5 are living. We compared these results with 2 case control populations. First of all, we investigated the occurrence of solid tumors in the 117 individu- als (64 males, median age 10 years at time of marrow donation) who served as stem cell donors for HCT. One donor developed breast cancer 29 years after marrow donation at age of 38. The 30-yr CI of developing solid tumor for donors was 4.5±2.21% with a statistically significant difference (p=0.03) as compared to that of transplantated patients. The second case control population consisted of 117 healthy individuals affected by cancer (median age 50 years). As previously, the matching technique applied was based on the variables age and sex. One control per case (transplanted patient) was randomly selected from the MIOT (Myocardial Iron Overload in Thalassemia) registry and matched by sex and age with the transplanted patient population. Two patients developed an hepatocellular carci- noma (HCC) at age of 39 and 44 years, respectively. One patient died and one is living. Using the event rate measure, we observed an event rate of 0.102 at 30 years for the transplant group and 0.041 for the nontransplant group (p=0.106).

Summary/Conclusions: This study shows that the magnitude of increased risk of SST is twofold to threefold for patients treated with HCT as compared with age- and sex-matched nontransplant TM patients or with stem cell donors. Notably, among the transplanted patients we didn’t observe any case of HCC, which is one of the most frequent solid tumor in nontransplant TM patients, whereas we observed 4 cases of head/neck cancers. In our series, cGVHD seems to be a strong risk factor in the development of new solid tumors. Patients with cGVHD, especially those with involvement of the oral cavity, must receive a very long careful monitoring and surveillance in order to prevent the development of secondary cancers.

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VALIDATING A NOVEL CAPILLARY ELECTROPHORESIS: THE MOST SUITABLE PLATFORM FOR THE NATIONAL NEWBORN SCREENING DEVELOPED IN A REGION WITH A HIGH PREVALENCE OF THALASSEMA AND HEMOGLOBINOPATHIES

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1136 | haematologica | 2017; 102(2)
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 targeting the thalassemias 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 >98% of abnormal globin alleles found in Thailand using α-thal GAP-PCR, α-thal ARMS-PCR, β-thal ARMS-PCR, and PCR-RFLP for Hb E. We compared CE data with each globin genotypes and use a ROC curve to set up new diagnostic criteria using% Hbs from CE for future cases.

Results: Identification of Hb Bart’s provided 100% of sensitivity, specificity and accuracy in most individuals with α-thal. Using ROC analysis, we proposed different cut-off values of Hb Bart’s to differentiate Hb H disease, α-thal and non-deletional α-thal traits; ≥7.40%, ≥0.85%, ≥0.45%, respectively with excellent accuracy (Table 1). Interaction of Hb E with these α-thal genotypes has no effect on these cut-off values (Table 1). However, there was a limitation to identify deletional α-thal Bart’s ≥20.10% (detectable level). A cut-off level to distinguish Hb EE from Hb E trait was suggested at ≥4.95% vs ≥0.7%.

Two patients with Hb E/β-thalassemia were identified through this study with different CE pattern from Hb EE. Thirteen β-thalassemia traits were identified and they had a lower level of Hb A as compared to their gestational age (GA) sex matched controls with normal β globin genotypes (68%). We recommend Hb A level ≤10.35%; a cut-off to primarily consider for β-thalassemia 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 practice into a routine program on prevention and control for severe thalassemia carrier followed by molecular analysis.

Table 1.

<table>
<thead>
<tr>
<th>Hb Trait</th>
<th>CE Cut-off (Linearity%)</th>
</tr>
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<tbody>
<tr>
<td>Hb Bart’s (detectable)</td>
<td>≥20.10%</td>
</tr>
<tr>
<td>Hb Bart’s (neutral)</td>
<td>≥7.40%</td>
</tr>
<tr>
<td>Hb Bart’s (low)</td>
<td>≥0.85%</td>
</tr>
<tr>
<td>Hb Bart’s (high)</td>
<td>≥0.45%</td>
</tr>
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</table>

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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 over-load. 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 at 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 splenome- tomized, 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, mean LIC 6.68±5.37 mg/g dw, ferritin 700±596 nmol/ml, Hb 9.3±1.3 g/dl, ALT, AST GGT and ALP were normal. LSM correlate with GGT (p <0.01) and AST values measured ≤10yrs (p=0.02). LIC correlate with ferritin (p=0.02). Seven/101 (6.9%) patients were HCV RNA positive, one of them had never been transfused. At baseline 37/101 (36.6%) patients were on iron chelation (ICT) (29 deferroxamine, 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 tests; only GGT (mean GGT 1.7±1.0 U/L in 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.

Summary/Conclusions: NTDT patients could benefit from regular non-inva- sive assessment of liver fibrosis. In our study subject who received ICT had best chance to reduce the grade of fibrosis through the reduction in iron overload. These patients, that usually are HCV RNA negatives, monitoring and treating iron overload is a crucial point in the prevention of hepatic siderosis causing the hepatic siderosis the primary cause of hepatic tissue damage, cirrhosis and hepatocellular carcinoma.

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

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Background: The introduction of close monitoring, regular blood transfusions and systematic iron chelation in the management of thalassemia have signifi- cantly changed the clinical phenotype of the patients and improved their sur- vival. The patients, who have benefited of the current therapeutic regimen, are now reaching middle age, and they have started facing problems commonly seen in older people. Recent observations suggest of an increased inci- dence of malignancies in the aging group of thalassemic patients.

Aims: The purpose of this study is to determine the longitudinal changes in the incidence of malignancies, along with possible correlations to different aspects of the disease.

Methods: A retrospective study in the largest Thalassemia Unit in Greece has been conducted spanning an observation period from 2001-2016. The occur- rence and type of cancer, as well as history on transfusion dependence, liver failure, HCV infection and chelation therapy were recorded and analyzed. Sta- tistical tests were performed using SPSS software package, v. 20. A p-val- ue<0.05 was considered statistically significant.

Results: Records from 591 patients (338 with thalassemia major and 253 with thalassemia intermedia) were evaluated. 27 patients (11 males and 16 females)
with malignancies were identified (incidence: 4.6%). The mean age of the diagnosis of the malignancy was 41.6 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 (1.8%). Liver cancer had the highest incidence 29.6%, followed 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 statistic 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% at 11-14 years of age and 12.8% at 0-5 years of age, 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 (p=0.118). As the utilization of different types of chelation changed throughout the years, according to the availability of the chelating agents, we analyzed separately, the patients that developed malignancies in the period after 2010 when longitudinal exposure to all three chelators can be assumed. Even though the results showed a difference (p=0.027) between the different groups with 47.1% of those patients receiving DFX at the time of diagnosis compared to 27.1% receiving DFP and to 11.8% receiving DFO, this distribution reflects the overall distribution of chelator usage during that period. Apart from the incidence, there was no statistical significant difference between TD and NTD patients with cancer regarding the gender, age and year of diagnosis. The cancer overall 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 neoplasm.

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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 deferiprone in minimally transfused pediatric thalassemia patients and to evaluate if it can postpone iron overload in these group of patients.

Methods: In the current trial (ClinicalTrials.gov Identifier: NCT02173951), 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) ≥0.6 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 ng/mL were withdrawn from the study and placed on a standard chelation regimen). Complete blood count was done weekly in DFP treated and every 3-4 weeks in NC.

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

Table 1.

<table>
<thead>
<tr>
<th>Time</th>
<th>SF (µg/L)</th>
<th>DFP</th>
<th>NC</th>
<th>DFP</th>
<th>NC</th>
<th>DFP</th>
<th>NC</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 months</td>
<td>43-95</td>
<td>94</td>
<td>93</td>
<td>43</td>
<td>93</td>
<td>43</td>
<td>93</td>
</tr>
<tr>
<td>6 months</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>9 months</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>12 months</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
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</table>

All NC patients were removed from the trial prior to completing 7 months of follow-up (9-11 transfusions) due to confirmed SF ≥1000 µg/L. Mean ± SD time of follow up was 10.4±4.9 and 5.9±2.5 months for DFP and NC respectively. Most common adverse events in patients on DFP versus NC were diarrhea (19% vs 13%, p=0.73), vomiting (13% vs 13%, p=1.00), abdominal colic (13% vs 13%), elevated liver enzymes (6% vs 3%, p=1.00) and neutropenia (6% vs 6%). All adverse events were mild in severity and did not require interruption of DFP use. There were no cases of agranulocytosis or moderate neutropenia, no arthralgia and no serious infections in DFP-treated patients. DFP therapy was associated with a significant reduction in the rate of iron accumulation as measured by SF (P<0.0001), LPI (P<0.001) and TSAT (P<0.001) (Figure 1a, b, c). LPI≥0.6 µM appeared as early as after 5 transfusions in NC children and was delayed to at least 10 transfusions with DFP therapy. TSAT≥70% appeared after 10 transfusions in NC children and was delayed to at least 17 transfusions with DFP therapy. The results of this study show that LPI and TSAT may reach values ≥0.6 µM and ≥70%, respectively, after 5-10 transfusions in children with TM and all NC children had SF ≥1000 µg/L after 8-9 transfusions.

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LONGITUDINAL PROSPECTIVE MRI STUDY IN PEDIATRIC PATIENTS WITH THALASSEMA MAJOR

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Background: No studies are available in literature evaluating, on repeated assessments, changes in myocardial and hepatic iron overload, biventricular function, and development of macroscopic myocardial fibrosis in pediatric patients with thalassemia major (TM).

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

Methods: We considered 68 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) project with less than 18 years at the first MRI scan and who performed a follow-up (FU) study at 18±3 months. Myocardial and hepatic iron burdens were quantified by the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: At the baseline MRI, 16 (23.5%) patients showed myocardial iron overload (MO: global heart T2*<20 ms) and 54 patients liver iron overload.
(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.

Methods:

Dec16. MRI-R2* heart, liver and pancreas in a cohort of well treated TM patients. Results: 69 TM patients: 43% male, age 38±9yrs, median number of observa-
tions/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%), deferasirox (basal 45%>f.u.52%), daily alternating deferasirox+deferiprone (basal 3%-f.u.6%), deferoxamine (basal 9%>f.u.6%) deferoxamine+

(p=0.017) and pancreas (p=0.018). Once again we found a correlation between the values of ferritin and R2* of liver (R2=0.37,p=0.0015), heart (R2=0.26, p=0.028) and pancreas (R2=0.23,p=0.05). Moreover the variations of ferritin correlate with the variations of R2* of the liver (R2=0.6,p<0.001), heart (R2=0.25, p=0.04) and pancreas (R2=0.41,p<0.001). Finally, assuming the cutoff value of 100 Hz for the pancreatic-R2* as the predictor of a cardiac R2*>50Hz, we calculated the numbers of false/true positive/negative according to the rule above. At the baseline we can observe that the number of false positive is the 14/27 (52%). The percentage increases to 91% (21/23) after f.u.: the pancreas-
R2*>100Hz in 23 patients but only 2 has iron overload in the heart; the total number of patients with pancreatic-R2*>100Hz is quite the same before and after f.u. (27 compared to 23). We found no correlation between the false pos-
itve 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 mul-
ti-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).

### Table 1.

<table>
<thead>
<tr>
<th>R2* (Hz)</th>
<th>Heart</th>
<th>Liver</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 50</td>
<td>False Positive</td>
<td>True Positive</td>
<td>False Negative</td>
</tr>
<tr>
<td>50-100</td>
<td>21</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>&gt; 100</td>
<td>1</td>
<td>3</td>
<td>23</td>
</tr>
</tbody>
</table>

Figure 1.

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-TALASSEMAIA 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 and 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%), deferasirox (basal 45%>f.u.52%), daily alternating deferasirox+deferiprone (basal 3%-f.u.6%), deferoxamine (basal 9%-f.u.6%) deferoxamine+deferiprone (basal 13%>f.u.4%). The observation at the baseline showed a positive strong correlation between R2* values of pancreas and both of liver (R2=0.68, p<0.001) and heart (R2=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, so this parameter should be considered with caution in the tuning of the 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**

T. Mizukami1, K. Nojima1, R. Sobata2, W. Kuriyashii3, C. Matsumoto4, Y. Yamasaki5, E. Sasaki1, K. Furuhata1, Y. Hiradate1, K. Ohkuma1, S. Matsuoka1, M. Satake1, I. Hamaguchi1

1Department of Safety Research on Blood and Biologicals, National Institute of Infectious Diseases, 2Central Blood Institute, Japanese Red Cross, 3Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, Tokyo, Japan

**Background:** Adult T-cell leukemia (ATL) is a malignant disease caused by infection with human T-lymphotropic virus type 1 (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-IG purified from the passenger carrier with a proviral load (PVL) 4 × 10^6/kg inhibited both HTLV-1 infection and syncytia formation. We purified HTLV-IG from the HTLV-1 positive plasma (PVL 4 × 10^6/kg) and evaluated its effect in a humanized mouse model. NOG (NOD.Cg-Fkdcscid Il2rtm1Sug/Cic) mice were treated with HTLV-IG for 5 days before HTLV-1 infection. During the monitoring period of 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 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 assay. We next assess the viral safety of HTLV-1 during the HTLV-IG manufacturing process. High log reduction values of HTLV-1 can be seen during the Cohn fractionation process. Virus safety was assessed with PCR based assay and in vitro and vivo infection assay.

**Summary/Conclusions:** These data suggest HTLV-IG is effective and safe for the prevention of HTLV-1 infection.

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**P402**

**THE COMBINATION OF TUMOR CELLS IN THE APHERESIS MATERIAL DOES NOT PREDICT THE RESPONSE OF MULTIPLE MYELOMA PATIENTS TO AUTOLOGOUS TRANSPLANTATION**

M. L. Lozano1, F. De Arriba1, M. Sola1, A. Sanchez-Fuentes1, N. Revilla1, F. Orduño1, A. Jerez1, I. Heras1, P. Iniesta1, O. Lopez-Godino1, M. D. Garcia-Malo1, V. Vicente1

1Hematology and Clinical Oncology, Hospital Universitario Morales Meseguer, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CB15/00555-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.

**Methods:** Patients (n=114) undergoing ASCT (n=120) for MM between January 2003 and February 2016 were enrolled in a retrospective study to analyze the prognostic value of aberrant (CD38++CD138+CD19-CD45weak) to normal phenotype (CD38++CD138+CD19+CD45+) plasma cells (ATC PC ratio) in the autograft by flow cytometry. The Durie-Salmon stage at diagnosis, response of disease to induction treatment, biological parameters, pre-ASCT percentage of PC in bone marrow and at day +100, and the mobilization of PC contained in the autograft of patients with a better vs poorer pre-ASCT response (0.08 vs 0.08 ×10^6/kg). There was no difference between the type of mobilization (G-CSF vs chemotherapy+G-CSF) and the degree of apheresis contamination (median A:T:PC ratio 0.5 vs 0.8; P=0.86). There was a statistical trend between the degree of infiltration of PC in the bone marrow before ASCT and the detection of atypical PC in the graft (p=0.06). At day +100, 94% of patients with CR or VGPR to induction therapy maintained the response, and 49% of patients in PR, SD or PD achieved post-ASCT CR or VGPR (p=1.24^-7). There was no association between the content of atypical PC in the graft and the response to day +100. However, the percentage of pre-ASCT PC in the bone marrow was significantly related to the response at day +100 (CR or VGPR vs PR, SD or PD), p=0.003, as well as the pre-ASCT monoclonal component (p=4.03^-7).

**Table 1.**

<table>
<thead>
<tr>
<th>Median age, yr (range)</th>
<th>60 (36-70)</th>
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</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>55 (45.8)</td>
</tr>
<tr>
<td>Plasma cell dyscrasia, n, %</td>
<td></td>
</tr>
<tr>
<td>- Multiple myeloma, IgG</td>
<td>66 (55.0)</td>
</tr>
<tr>
<td>- Multiple myeloma, IgA</td>
<td>28 (23.3)</td>
</tr>
<tr>
<td>- Bence-Jones multiple myeloma</td>
<td>15 (12.5)</td>
</tr>
<tr>
<td>- Plasma cell leukemia</td>
<td>5 (4.2)</td>
</tr>
<tr>
<td>- Other: Non secretory myeloma</td>
<td>6 (5.0)</td>
</tr>
</tbody>
</table>

**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.

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**P403**

**EVALUATION OF THERAPEUTIC PLASMA EXCHANGE AT A TERTIARY LONDON HOSPITAL**

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**Background:** Therapeutic plasma exchange (TPE) is used to treat a number of haematological, renal and neurological conditions. Pathogenic antibodies or other plasma molecules are removed, and plasma volume is replaced with fluid. Human albumin solution (HAS) is usually preferred, except in cases of Thrombotic Thrombocytopenic Purpura (TTP) and related conditions. TPE may result in dilutional coagulopathy, and reactions such as hypersensitivity can occur. The British Society for Haematology (BSH) published a 2015 guideline to assist the use of TPE in UK clinical practice, providing evidence-based indications and recommended schedules.

**Aims:** To evaluate the use of elective TPE at a large tertiary London hospital, compare clinical practice against BSH guideline recommendations, and explore the effect of TPE on coagulation test results.

**Methods:** Data was collected prospectively over a 2 month period, using patient notes and electronic transfusion records. A data collection form recorded the indication, treatment schedule, replacement fluid, complications, the presence of a written treatment plan, and frequency and results of coagulation testing.

**Results:** 24 plasma exchanges took place over the period of data collection; there were no cases of TTP. Adherence to BSH were variable; although most cases (88%) had an evidence-based clinical indication for TPE, just 4% had a full written treatment plan, and only 17% of courses followed recommended scheduling. 75% of patients had received at least one prior course, some outside guideline indications for repeat courses. Most patients (83%) initially received HAS, however 87% received FFP at some point during TPE, with 42% receiving Solvent Detergent FFP. In 17% of patients this fluid change was due to a reaction, but for the remainder it was due to dilutional coagulopathy. The guidelines recommend fibrinogen monitoring, and although most patients had baseline measurement (75%), subsequent testing showed wide variation. Despite this, 71% had a fibrinogen of 1 g/l measured during TPE. Fibrinogen levels showed some correction by the next day but usually still abnormal. A prolonged aPTT and PT was also seen in most patients immediately following TPE, which almost always corrected by the next day.
A COMPREHENSIVE PROTEOMICS STUDY ON PLATELET CONCENTRATES: PLATELET PROTEOME, STORAGE TIME AND MIRASOL PATHOGEN REDUCTION TECHNOLOGY

V. Saiappan, F. Seiguer, A. M. Granderath, P. C. O'Gara, C. Bon, M. I. Sivakumaran, M. Platt

Background: Platelet concentrates (PCs) represent a blood transfusion product with a major concern for safety as their storage temperature (20-24°C) allows bacteria to grow. We aimed to identify changes in protein levels less than 7 weeks from complete microbiological testing. Pathogen reduction technologies (PRTs) provide an additional layer of safety to the blood transfusion products from known and unknown pathogens (such as bacteria, viruses and parasites). In this context, PRTs (such as Mirasol technology) have been developed and are implemented in many countries. However, several studies have shown in vitro that Mirasol PRT induces a certain level of platelet shape change, hyperactivation, basal degranulation and increased oxidative damage during storage. It has been suggested that Mirasol PRT might accelerate what has been described as the platelet storage lesion (PSL), but supportive molecular signatures have not been obtained.

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

Methods: We present comprehensive proteomics data analysis of control PCs and PCs treated with Mirasol PRT at storage day 2, 6 and 8. Our workflow was set to perform proteomics analysis using a gel-free and label-free quantification (LFQ) approach for which two replicate samples were taken. To identify and quantify the altered proteins, we used MaxQuant/Perseus software platform.

Results: We identified marginal differences between Mirasol PRT and untreated PCs during storage. However, those significant changes at the proteome level were specifically related to the functional aspects previously described to be a methodology suitable to phenotype platelets in an unbiased manner, in various physiological contexts.

Summary/Conclusions: In summary, semi-quantitative proteomics allows to discern between treatment changes due to Mirasol PRT or PSL, and proves to be a methodology suitable to phenotype platelets in an unbiased manner, in various physiological contexts.

USE OF A SURVEY TO ASSESS AND IMPROVE ADHERENCE TO UK BLOOD TRANSFUSION GUIDELINES IN A HOSPITAL SETTING

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Background: UK guidelines to provide evidence-based support for decisions to transfuse packed red cells were published in 2015 by NICE (National Institute for Health and Care Excellence). The guidelines specified hemoglobin (Hb) targets for transfusion, use of single unit transfusion to avoid over-transfusion, information provision to patients for informed consent, and avoidance of pre-operative transfusion by timely identification of iron deficiency for referral through an anemia pathway. A local baseline audit of NICE compliance at our London tertiary referral hospital showed low overall compliance with these recommendations.

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

Methods: An online survey, designed to both evaluate and inform participants, was targets at doctors of different training grades and specialties during a two week period. The outcomes of this are being used to guide further training.

Results: Of 141 participants who took part in the survey, 31% (43) had been qualified for less than two years and 47% (65) were consultants. Specialities included Surgery, Anesthetics, Internal Medicine, Hemato-Oncology and Intensive Care. 60% (84) had prescribed blood within the last month. Despite only 51% (72) awareness of the NICE guidelines, a significant majority (73%, 103) selected the correct Hb threshold of 57g/L for transfusion in patients without acute coronary syndrome (ACS). A lower threshold of 50g/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 in only 45% (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 endorsed treatment indication for transfusion, and 90% (127) gave an opportunity to ask questions and ensured the patient was content to proceed. Provision of written information was poor (26%, 37) and only 55% (78) recorded the discussion in patients’ notes. Exploring barriers to consent, 24% (32) expressed difficulty in obtaining a patient information leaflet, and issues relating to lack of time and 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 increased their awareness, and as such a high response rate, 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 to improve the hospital transfusion templates which were distributed to all hospital staff, and carrying out structured case based discussion sessions with junior doctors to enhance knowledge and confidence.

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

M. Healey1,*, M. Sivakumaran2, M. Platt1

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Background: Prion diseases are a group of fatal transmissible neurological conditions whose disease etiology is characterised by the change in conformation of the normal intrinsic cellular prion protein (PrPc) to in the highly ordered insoluble amyloid state conformer (PrPSc). The significant event fundamental to the progression of these diseases is the self-catalytic, and perpetuating, nature of the conversion of PrPc in the presence of PrPSc aggregates. The disease is characterized by the prion protein conformational changes in the United Kingdom during the 1990s, is considered to be an effect of dietary exposure to the bovine spongiform encephalopathy (BSE) agent through contaminated meat products. To date, the widely accepted estimate for the prevalence of vCJD in the UK puts the number of potential cases at 1 in 2000. Since the disease is known to be infectious and transmissible, the iatrogenic ability of this disease is a significant risk to public health through transfusion products and surgical procedures.

Aims: We aim to develop a reliable and robust assay that can be used as a screening tool to detect the infectious PrPSc protein at low levels in human blood with high selectivity and high sensitivity.

Methods: Here we use a technique based on the Coulter Counter principle that uses tunable elastomeric nanotubes termed Tunable Resistive Pulse Sensing (TRPS) to detect the prion protein without an amplification step. The first stage optimises the grafting of an ssDNA aptamer onto nanoparticles. In proof of concept work, the functionalized nanoparticles were used to detect the cellular prion protein in phosphate buffered saline by monitoring the relative change in velocity through the nanopore, which is then converted to zeta potential. The method was then applied to protein rich samples and serum.

Results: By varying the concentration of aptamer relative to the binding capacity of the nanoparticles, a sigmoidal binding curve was observed. Here mean zeta values were -1.94 mV for 0%; -4.43 mV for 33%; and -7.30 mV for 100%. The assay was further developed by monitoring the functionalized particle’s translocation velocity as a function of prion protein concentration. Increasing the concentration of the protein caused shielding of the polyanionic DNA by the positive protein at pH 7.4, therefore the velocity of the negatively charged particle conjugate decreased. The lowest concentration to have a significant change (p=0.05) in velocity distribution was 1 nM, with a 2.5% decrease relative to 0 nM. The higher concentration of 50 nM had a bigger effect of 24% decrease.

Summary/Conclusions: TRPS technology presented here offers the ability to rapidly and accurately detect the presence of prion protein by particle assay design. Any relative change to the functionalized particle’s signal could be observed, demonstrating its capability and suitability to detect biological targets.
Front-line combinations in multiple myeloma and amyloidosis

**S407 QUADRUPLET VS SEQUENTIAL TRIPLET INDUCTION THERAPY FOR MYELOMA PATIENTS: RESULTS OF THE MYELOMA XI STUDY**


1The Institute of Cancer Research, London, United Kingdom, 2Myeloma Institute, University of Arkansas for Medical Sciences, Little Rock, United States, 3Clinical Trials Research Unit, Leeds Institute of Clinical Trials Research, Leeds, 4Heart of England NHS Foundation Trust, Birmingham, 5Leicester Royal Infirmary, Leicester, 6Centre for Clinical Haematology, Nottingham University Hospital, Nottingham, 7University Hospitals of North Midlands, Stoke-on-Trent, 8Kent and Canterbury NHS Trust, Canterbury, 9Southampton Hospital, Southampton, 10University of Leeds, Leeds, 11The Royal Marsden Hospital, London, 12Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, 13Haematological Malignancy Diagnostic Service (HMDS), St James’s University Hospital, Leeds, 14Clinical Trial Research Unit, Leeds Institute of Clinical Trials Research, Leeds, 15Department of Haematology, Newcastle University, Newcastle, United Kingdom

**Background:** Combining anti-myeloma induction therapies limits the impact of clonal heterogeneity on resistance to therapy, maximising response and associated clinical outcomes. Triple 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 therapy for all patients by the use of quadruplet combinations upfront.

**Aims:** The UK NCRI Myeloma XI trial is a large, phase III study comparing, in transplant eligible (TE) patients, the induction quadruplet carfilzomib, cyclophosphamide, lenalidomide and dexamethasone (KCRD) to the sequential strategy of triplet immunomodulatory combinations (with thalidomide or lenalidomide) followed by additional pre-transplant consolidation with PI triplet therapy for those with a suboptimal response.

**Methods:** In 2013, the TE pathway of the Myeloma XI study was amended to include KCRD given in 28 day cycles (carfilzomib 36mg/m² IV d1-2,8-9,15-16 (20mg/m² #1d1-2), cyclophosphamide (cyclo) 500mg PO d1, lenalidomide (len) 25mg PO d1-21, dexamethasone (dex) 40mg PO d1-4,8-9,15-16). Patients were randomised to this up-front quadruplet or the sequential strategy of CRD (cyclo 500mg PO d1, len 25mg PO d1-21 PO daily, dex 40mg PO d1-4,8-9,15-16) or CTD (cyclo 500mg PO d1,8,15 thalidomide 100-200mg PO daily, dex 40mg PO d1-4,12-15) given to max. response. Patients with VGPR or CR proceeded straight to ASCT, those with PR/MR were randomised to sequential CVD (cyclo 500mg d1,8,15, bortezomib 1.3mg/m² IV/sc d1,4,8,11, dex 20mg PO d1,2,4,5,8,9,11,12) or nothing and those with SD/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>
<thead>
<tr>
<th>Table 1. Treatment exposure and safety data.</th>
</tr>
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<tbody>
<tr>
<td><strong>Response at end of first induction therapy</strong></td>
</tr>
<tr>
<td>CTD (n=1021)</td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>VGPR</td>
</tr>
<tr>
<td>PR</td>
</tr>
<tr>
<td>VGPR</td>
</tr>
<tr>
<td>VGPR at day 100 after ASCT (number completing ASCT to date)</td>
</tr>
<tr>
<td>CTD (n=647)</td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>VGPR</td>
</tr>
<tr>
<td>PR</td>
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</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 receive a minimum of 4 cycles of initial induction with therapy continued to maximum response. The median number of cycles delivered was CTD: 5, CRD: 5, KCRD: 4. Grade ≥3 haematological toxicities differed between the groups. (Neutropenia CTD: 12%, CRD: 22%, KCRD: 16%; Thrombocytopenia CTD: 3.4%, CRD: 4.5%, KCRD: 8.1%; Anaemia CTD: 6.7%, CRD: 9.6%, KCRD 10%). Grade ≥2 neurological toxicity was greater with the thalidomide-containing regimen (Sensory neuropathy CTD: 9.5%, CRD: 3.4%, KCRD: 2.3%). There was no statistically significant difference in rates of investigator reported, all-grade, thromboembolic events between regimens (CTD: 11.8%, CRD 11.1%, KCRD 14.7%). Response to initial induction and following ASCT is shown in Table 1 indicating deeper responses with the quadruplet compared to triplets both at the end of first induction regimen (p<0.0001) and, importantly, post-ASCT (p<0.0001). These differences were observed despite the use of randomised pre-transplant consolidation for suboptimal responders to triplet immunomodulatory therapy.

**Summary/Conclusions:** Induction therapy with KCRD, an outpatient delivered quadruplet regimen, was associated with deeper responses than immunomodulatory triplet therapy (CRD/CTD) and was well tolerated. Deeper responses persisted after ASCT, with an impressive response rate ≥VGPR of 92% with KCRD.

**S408 DEEP AND DURABLE RESPONSES WITH WEEKLY IXAZOMIB, LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA: LONG-TERM FOLLOW-UP OF PATIENTS WHO DID NOT UNDERGO SCT**

S. Kumar1,*, J. Berdeja2, R. Niesvizky3, S. Lonial4, J. Laubach5, M. Hamadani6, S. Kumar1,*, J. Berdeja2, R. Niesvizky3, S. Lonial4, J. Laubach5, M. Hamadani6,1 The Institute of Cancer Research, London, United Kingdom, 2Myeloma Institute, Weill Cornell Medical College, New York Presbyterian Hospital, New York, 3Department of Immunology and Medicine, Winship Cancer Institute of Emory University, Atlanta, 4Dana-Farber Cancer Institute, Boston, 5West Virginia University, Mary Babb Randolph Cancer Center, Morgantown, 6Mayo Clinic College of Medicine, Scottsdale, 7Division of Hematology Oncology, Medical College of Wisconsin, Milwaukee, 8Mayo Clinic, Jacksonville, 9Cedars-Sinai Outpatient Cancer Center at the Samuel Oschin Comprehensive Cancer Institute, Los Angeles, 10Winship Cancer Institute of Emory University, Atlanta, 11Millennium Pharmaceuticals Inc., Cambridge, MA, USA, a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, United States

**Background:** Triplet combinations that include a proteasome inhibitor (PI) have been proven superior to doublets in newly diagnosed multiple myeloma (NDMM) (San Miguel et al, N Engl J Med 2008, Durie et al, Lancet 2017). The all-oral combination of the novel PI ixazomib plus lenalidomide-dexamethasone (IRD) was evaluated as an induction regimen in NDMM patients, followed by single-agent ixazomib maintenance.

**Aims:** Here we report updated efficacy and long-term safety data for patients who did not withdraw from the study in order to receive stem cell transplantation (SCT).

<table>
<thead>
<tr>
<th>Table 1.</th>
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<tbody>
<tr>
<td>All patients who did not withdraw to receive SCT (n=42)</td>
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<td></td>
</tr>
<tr>
<td>Maintenance subset (n=23); AE's with onset during induction (cycles 1-12)</td>
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<td></td>
</tr>
<tr>
<td>AE's with onset during maintenance (cycles 13-24)</td>
<td></td>
<td></td>
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<tr>
<td><strong>Treatment exposure</strong></td>
<td></td>
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<tr>
<td>Median number of treatment cycles (range)</td>
<td>17 (1-73)</td>
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<tr>
<td><strong>Safety summary</strong></td>
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Methods: In this phase 1/2 study (NCT01217957), patients with NDMM received weekly oral ixazomib (1.68-3.95mg/m²; days 1, 8, and 15) plus lenalidomide (25mg, days 1-21) and dexamethasone (40mg, days 1, 8, 15, and 22) for up to twelve 28-day induction cycles, followed by maintenance therapy with weekly single-agent ixazomib, at the last tolerated dose given during induction, until disease progression or toxicity.

Results: Of the 65 enrolled patients, 42 continued on study treatment without early withdrawal for SCT; the long-term follow-up of these 42 patients is reported here. Baseline patient characteristics included: median age, 68 years (range 34-86); ISS stage I/II/III in 40%/43%/17%. As of October 18, 2016, with median follow-up of 56 months, the confirmed overall response rate (ORR; ≥ partial response [PR]) in patients 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. Three-year OS rate was 87%. Safety findings are summarized in the Table; 74% of patients had grade ≥3 treatment-related adverse events (AEs), and 26% of the patients had treatment-related serious AEs. Among treatment-related AEs of interest, grade ≥3 rash and peripheral neuropathy were infrequent. There was one treatment-related death due to respiratory syncytial viral pneumonia. After completing 12 cycles of induction therapy with IRd, 25 patients went on to receive maintenance single-agent ixazomib. In these 25 patients, at the end of the induction period ORR was 100%, including 44% VGPR and 32% CR. Responses deepened during maintenance; at data cut-off, the response rates in this maintenance therapy population were 95% VGPR 32%, and CR 32%. Patients who received maintenance therapy was 24 months. The occurrence of the most common treatment-related grade ≥3 AEs (neuropenia, thrombocytopenia, and fatigue) was confined almost exclusively to the induction period. During the maintenance period no patients reported onset of grade ≥3 peripheral neuropathy or rash.

Summary/Conclusions: In patients with NDMM, weekly ixazomib plus Rd, followed by single-agent ixazomib maintenance, was highly active, resulting in deep and durable responses, long PFS, and a high 3-year OS estimate. IRd followed by single-agent ixazomib maintenance also showed an acceptable safety profile, with less toxicity reported during the maintenance (single-agent ixazomib) vs induction (IRd) periods, with no evidence of cumulative toxicities.
Summary/Conclusions: Safety profile was acceptable; more patients required plerixafor in the KRd arm. Rate of VGPR was higher with KRd. Updated data on a higher number of patients will be presented at the meeting. The trial is registered at Clinicaltrials.gov: NCT02203643

S411
HOVON 104; FINAL RESULTS FROM A MULTICENTER, PROSPECTIVE PHASE II STUDY OF BORTEZOMIB BASED INDUCTION TREATMENT FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH DE NOVO AL AMYLOIDOSIS

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Background: Bortezomib (B) has been reported to be very effective in AL amyloidosis with overall response rates (ORR) varying between 50-80%. However, there are no prospective data from multicenter studies on B treatment in de novo patients. We investigated the efficacy and safety of B-Dexamethasone (BD) induction treatment followed by HDM+SCT in de novo AL amyloidosis patients.

Aims: The primary aim was to improve the hematological CR rate at 6 months after SCT on intention to treat analysis from 30 to 50%. Secondary aims were OS, PFS, hematological response rate after BD treatment, organ responses, safety and prognostic factors for survival.

Methods: Patients with biopsy proven AL amyloidosis, aged between 18-70 years, with detectable M-protein and/or level of involved FLC>50mg/L, WHO performance status 0-2, NYHA stage I-II, 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 was 1 in 56% and 2 in 42% of patients. Mayo cardiac risk score was 1 (30%), II (36%), III (34%). Organ involvement was 82% renal, 66% heart, 28% liver, 14% neurological, 8% gastrointestinal and 38% of patients had 3 or more organs involved. Bone marrow plasmacells were >10% in 28% of patients. The median FU for patients alive is 24 (10-55) months. Twelve of 50 (24%) patients could not proceed to SCM. Four patients due to B related toxicity, 3 patients did not fulfill criteria to proceed, 2 patients died (both amyloidosis related) and 3 miscellaneous. Of these 38 patients, 3 went subsequently off protocol because of 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). Brentuximab vedotin (BV) treatment. The multicohort phase 2 CheckMate 205 trial (NCT02181738) enrolled pts with RR cHL after ASCT. Initial analyses revealed high objective response rates (ORR), encouraging duration of response (ORR) and an acceptable safety profile (Younes A et al, Lancet Oncol 2016). Durable responses to therapy are valuable in pts with progressive disease after failure of ASCT due to their limited treatment options.

Aims: To report extended follow-up data for all pts with RR cHL after failure of ASCT in CheckMate 205.

Methods: This is a single-arm multicenter trial enrolled pts (age ≥18 y) with RR cHL after ASCT into 1 of 3 independent cohorts (Cohort A: BV-naïve; Cohort B: BV only after ASCT; Cohort C: BV before and/or after ASCT). All pts received nivolumab 3mg/kg every 2 wk until disease progression or unacceptable toxicity.

- PR (≥15 mo in each cohort), and stable disease (≥9 mo in each cohort). Median follow-up was 19 (1, 25), 23 (2, 27) and 16 (1, 20) mo in Cohorts A, B, and C, respectively. DOR for patients with partial response (PR) was 17 and ≥11 months, respectively. PFS by cohort is shown (Figure 1). Prolonged median PFS was seen for patients with CR (≥17 mo in each cohort), PR (≥15 mo in each cohort), and stable disease (≥9 mo in each cohort).

Results: In total, 243 pts were treated: 63 in Cohort A (BV-naïve), 80 in Cohort B (BV after ASCT), and 100 in Cohort C (BV before [n=53], after [n=58], or before and after [n=52] ASCT). Median (range) age was 34 (18-72) y and 77% of pts had advanced (stage III+) disease at study entry. BV-naïve pts had fewer prior lines of therapy (median of 2 vs 4 with prior BV). At Dec 2016 database lock, median (min, max) follow-up was 19 (1, 25), 23 (2, 27) and 16 (1, 20) mo in Cohorts A, B, and C, respectively. Overall, 40% of pts were still on treatment; the most common reason for discontinuation was disease progression (26%). ORR was 65% in Cohort A, 68% in Cohort B, and 73% in Cohort C, with 29%, 13%, and 12% CR, respectively. Median (95% CI) DOR was 20 (13, 20), 16 (8, 20), and 15 (9, 17) mo in Cohorts A, B, and C, respectively. DOR for patients with CR was 20 months for BV-naive patients (Cohort A) and ≥15 mo for BV-treated patients (Cohorts B and C). DOR for patients with partial response (PR) was 17 and ≥11 months, respectively. PFS by cohort is shown (Figure 1). Prolonged median PFS was seen for patients with CR (≥17 mo in each cohort), PR (≥15 mo in each cohort), and stable disease (≥9 mo in each cohort). Median OS was not reached in any cohort. The most common drug-related AEs were fever (15%), injection 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 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


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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 suggested that escalated PET2 treatment to PET2 result could increase the efficacy of standard ABVD.

Aims: To confirm in a prospective setting the favorable prognosis of advanced stage PET2 negative patients treated with ABVD, as well as the safety and efficacy of escalated BEACOPP given to PET2 positive patients.

Methods: We conducted a prospective clinical trial (HD6067 ClinicalTrial.gov identifier 00795613), in which advanced-stage (IIb-IVh) 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 (BEACOPP plus BEACOPP baseline dose or BE+B or Be+B+ or rituximab (R)) PET2- (5-PS 1-3) to 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: After June 2014 (N=319), 191 PET2+ 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<0.0002). Out of 149 PET2+ patients randomized to Be+Bb (76) or Be+Bb+R (73), 136 were evaluable for response: 93 obtained CR and 43 had a treatment failure. Of the remaining 13 patients, 3 died, 7 withdrew their consent and 3 stopped treatment for toxicity. As per study protocol, 627 out of 630 PET2- patients continued with 4 ABVD cycles and 3 withdrew their consent. Overall, 30 patients (3.8%) died, due to early death (n=2), resistant disease (n=18; 12 with a positive and 6 with a negative PET2), transplant related toxicity (n=5), infections (n=4) and pulmonary fibrosis (n=1). After a median follow-up of 1303 days (2-2857), the 4-Y FFS 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 FFS was 69% (95% CI 60%>76%) and 87% (95% CI 84%>93%), 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 FFS of 69% (95% CI 57%>79%) and 68% (95% CI 55%>78%), 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 FFS of 95% (95% CI 91%>98%) for RxT and 93% (95% CI 87%>96%) for NFT (p=0.2882).

**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- patients presenting with large nodal mass; 4) the addition of Rituximab does not increase the effectiveness of Be+Bb in PET2+ patients.

**S414**

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

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**Background:** Data on disease presentation, therapeutic options and survival after 3rd or higher relapse of classical Hodgkin lymphoma (cHL) are sparse. Therefore the additional benefit of new agents, which were already initially investigated after several relapses of cHL, is difficult to estimate.

**Aims:** The aim of this study was to define and describe a historical control group in European patients from the German Hodgkin Study Group (GHSG) data for comparison of safety and efficacy of novel therapeutic agents.

**Methods:** Cases with at least three consecutive tumor-related events or progressive refractory or relapsed disease, were identified in the GHSG database. Detailed information was added from case report forms and physician’s letters. Overall survival (OS) was the main and progression free survival (PFS), response to therapy, adverse events, disease and treatment characteristics as secondary endpoints.

**Results:** Among 12,584 HL patients in the GHSG first-line trials HD7 to HD15 and 449 HL patients in the trials HDR1 and HDR2 a total of 69 cHL patients with ≥3 tumor events were identified. The dates of occurrence of 3rd relapse ranged between 15th of January 1993 and 21st of June 2013. The sample consisted of 51 male (74%) and 18 female (26%) patients. At time of 3rd relapse the age of the patients ranged from 20 to 79 years (mean 39.2 years, standard deviation (SD) 14.0 years) and the majority of patients presented with stage III or IV disease (67%). Time from end of 3rd-line treatment to 3rd relapse was ≤3 months (i.e. GHSG definition of refractory disease) in 15 cases (22%), ≤12 months (early relapse) in 19 cases (28%) and >12 months (late relapse) in 35 cases (51%). All 69 patients were pretreated with chemotheraphy, 35 (50.7%) with BEACOPP, 30 (43.5%) with ABVD and no BEACOPP, and 32 (46.6%) with another type of chemotherapy. The number of prior chemotherapies ranged from one to three (median 3). Pretreatment with radiotherapy was observed in 57 (82.6%) patients, with salvage chemotherapy aimed to induce a remission prior to a stem-cell transplantation (SCT) in 58 (84.1%), and with high dose chemotherapy followed by autologous SCT in 50 (72.5%) patients. Four patients (5.8%) had received allogeneic SCT as 3rd-line treatment. None of the patients had received brentuximab vedotin or anti-PD1 antibodies before 3rd relapse. With a median observation time of 63.3 months for OS after 3rd relapse, 45 patients (65.2%) had died and 60 (87.0%) had another PFS event. Twelve months after the 3rd relapse OS was 73.2% (95%-CI 62.6% to 83.8%) and PFS 50.8% (95%-CI 38.9% to 62.8%, Table 1).

**Table 1.**

<table>
<thead>
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<tr>
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<tr>
<td>12 months</td>
<td>59.8%</td>
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</tr>
<tr>
<td>18 months</td>
<td>54.3%</td>
<td>77.0%</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** Patients with a 3rd relapse or progression of cHL have a dismal, mostly palliative prognosis due to frequent tumor progression. Within one year half of the patients have a PFS event and one fourth die.

**S415**

**A REVISED STAGING SYSTEM FOR WALDENSTRÖM’S MACROGLOBULINEMIA**


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**Background:** Waldenström’s macroglobulinemia (WM) is a rare low-grade B-cell lymphoma characterized by the lymphoplasmacytic bone marrow infiltration and elevated levels of immunoglobulin M (IgM) monoclonal immunoglobulin. This 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 (IPSSWM) which was developed in 2009 based on data of patients that were treated primarily without rituximab and mainly with alkylators and nucleoside analogues. IPSSWM is based on five covariates (age, hemoglobin, platelet counts, IgM levels and b2 microglobulin) and stratifies WM patients into 3 broad risk groups. IPSSWM does not take into account non-IgM elevated mortality, which is common and quite different among patients over the age of 75 year and does not include LDH, which is a well identified prognostic factor both in lymphomas and multiple myeloma.
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 <11.5g/dl was common across all subgroups while low platelet counts <100x109/L was found in relatively few patients and had no prognostic significance. Based on ROC analysis for early death (within 3 years), serum albumin <3.5g/dl and b2microglobulin >4mg/L were the two most important prognostic factors of early WM-related death. Age >65 years was associated with increased risk of death, however, age >75 years conferred additional risk (double hazard of death compared to those 65-75 years and fourfold compared to patients <65 years). Thus, we formulated a score in which high b2 microglobulin, elevated LDH and low serum albumin are scored with 1 point each, age 66-75 years is scored with 1 point but age >75 years is scored with 2. As a result, patients with scores 0, 1, 2, 3 or 4-5 had 3-year WM-related death rate of 3%, 7%, 14%, 19% and 48% (chi-square: 80.7, p<0.001). Regarding overall survival, 10-year survival rate was 85%, 59%, 39%, 28% and 12% (p<0.001) (Figure 1). Because age is a major determinant of disposition we also evaluated this staging system in patients >65 years and retained it prognostic significance. Compared to IPSSWM, this new staging system outperformed ISSWM: c-statistics, a measure of performance of a prognostic tool, was 0.711 (95% CI 0.659-0.763) vs 0.652 for IPSSWM (95%CI 0.627-0.677) (p=0.008). 22 pts relapsed (6 of them with histologic transformation to DLBCL). 11/22 were retreated with R and 9/11 responded. 8 deaths were recorded: 3 of them disease related. R therapy was well tolerated. Only one pt could not complete treatment due to intolerance.

Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th># of cases</th>
<th>%</th>
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<td>Elevated LDH</td>
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<td>43</td>
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<tr>
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<td>SLSG prognostic system</td>
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<td>5-year PFS</td>
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<td>93%</td>
</tr>
<tr>
<td>10-year PFS</td>
<td>83%</td>
<td>83%</td>
</tr>
<tr>
<td>15-year PFS</td>
<td>88%</td>
<td>88%</td>
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<tr>
<td>10-year OS</td>
<td>93%</td>
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<tr>
<td>15-year OS</td>
<td>93%</td>
<td>93%</td>
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</tbody>
</table>

Summary/Conclusions: A revised staging system, based on b2 microglobulin, elevated LDH, low serum albumin and age identifies groups with very different outcomes among patients with symptomatic WM treated with contemporary regimens and may outperform IPSSWM.

S416 SPLENIC MARGINAL ZONE LYMPHOMA (SMZL) TREATED WITH RITUXIMAB (R) MONOTHERAPY: A LONG TERM FOLLOW-UP STUDY ON 104 PATIENTS

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Background: Rituximab monotherapy has been used successfully in the treatment of SMZL and it can replace splenectomy, at least in 1st line.

Aims: To present our data on the outcome of R monotherapy treated pts after a long term follow-up.

Methods: The diagnosis of SMZL was based on the WHO criteria. Criteria for treatment initiation included: bulky/symptomatic splenomegaly, cytopenias or presence of B-symptoms. All pts received 6 weekly cycles of R as 1st line therapy at a dose of 375mg/m2 (induction phase). None of the pts had been splenectomised before R treatment. Maintenance with R at a dose of 375mg/m2 every 2 months for 1-2 years was given according to physician’s discretion. Response assessment was based on the SLSG consensus criteria. Survival curves were estimated using the Kaplan Meier method and compared by log-rank test.

Results: 104 pts with SMZL were included. 45% were males with a median age of 66 y (41-91). At diagnosis all pts had bone marrow infiltration with a median % of infiltration of 40 (10-85). Anemia and thrombocytopenia were present in 30% and 19%, respectively. 40% had absolute lymphocytosis. LDH was elevated in 43%. According to the SLSG prognostic system, 39% were classified in group A, 56% in group B and 5% in group C. The median time from diagnosis to treatment initiation was 2 months (0-203). 71 pts received R maintenance. The overall response rate 2 months after the end of induction treatment was 93% (CR, CRu and PR in 42%, 21% and 30%, respectively). Maintenance therapy improved the quality of response in 19 of them, 52 pts maintained their initial response and one relapsed during maintenance phase. The 5- and 10-year PFS, OS and CSS were 70% and 64%, 93% and 88%, 99% and 93%, respectively. Maintenance therapy was associated with better PFS (p=0.008). 22 pts relapsed (6 of them with histologic transformation to DLBCL). 11/22 were retreated with R and 9/11 responded. 8 deaths were recorded: 3 of them disease related. R therapy was well tolerated. Only one pt could not complete treatment due to intolerance.

Figure 1.
Biology of MPN: JAK2 and beyond

S417

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

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Background: The JAK-STAT pathway is a critical controller of cellular proliferation, differentiation, survival and apoptosis in response to external stimuli. Promiscuous activation of this pathway is an important driver in the pathogenesis of BCR/ABL-negative chronic myeloproliferative neoplasms. The JAK2-V617F allele is the most common and characterized mutation linked to this class of leukemia. The increased activation of JAK-STAT signaling in JAK2-V617F cells can be partially explained by increased JAK2 autophosphorylation. It is unclear however if these effects are sufficient to fully account for the strong activation of the JAK-STAT pathway induced by JAK2-V617F. We recently described programmed -1 ribosomal frameshifting (-1 PRF) as a novel mechanism regulating the expression of ~10% of human genes, including cytokine receptors (Blewe AT et al., Nature, 2014). In this process, cis-acting mRNA elements (-1 PRF signals, which consist of a slippery site followed by a pseudoknot) direct translating ribosomes to slip by one base in the 3' direction, establishing a new reading frame. This directs ribosomes towards premature termination codons, resulting in destabilization of the -1 PRF signal-containing mRNA via nonsense-mediated mRNA decay (Figure 1). There is thus an inverse relationship between -1 PRF efficiency and mRNA stability.

Aims: To investigate whether the JAK2-V617F mutation, shown here to be located in the pseudoknot of a -1 PRF signal in the JAK2 mRNA, impacts disease progression through ablation of -1 PRF.

Methods: Computationally predicted -1 PRF signals were validated using dual luciferase reporters and proteomic analysis of a -1 PRF fusion protein. -1 PRF location codons, resulting in destabilization of the -1 PRF signal-containing mRNA, impacts disease progression through ablation of -1 PRF.

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 were reduced frameshifted, 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 V617Fm 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 (Bhagwat N et al, Blood, 2014).

S418

EFFECTIVENESS OF LSD1 INHIBITION FOR THE TREATMENT OF MPN

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Background: Treatment of MPN with JAK1 inhibitors ameliorates symptoms and splenomegaly but does not meaningfully reduce the JAK2V617F allele burden. Though curative, stem cell transplantation is associated with extensive morbidity and mortality highlighting the need for novel effective therapies. The histone ’lysine-specific demethylase 1A’ (LSD1/KDM1A) is being explored as a drug target in AML where LSD1 inhibitors already in the clinic are critical for sustaining self-renewal in leukemic initiating cells; inhibiting LSD1 induces monocytic differentiation and reduces engraftment of AML cell in vivo. LSD1 is over-expressed in a number of myeloid diseases including MPN. Preliminary data in a mutant patient model of MPN showed that a 28-day course of LSD1 inhibition had a beneficial impact on spleen size, cytokines and mutant cell burden. In a mutant JAK2 mouse model of MPN, we have characterized the disease-modifying activity of the LSD1 inhibitor (IMG-7289), a compound in clinical development for myeloid diseases (NCT02842827).

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

Methods: Jak2V617F-L2 mice were crossed to MxCRE mice and displayed a fulminant MPN phenotype without dIdC induction. CBC and BM FACS analysis were conducted as previously described. We designed a qPCR assay to quantify murine JAK2V617F allele burden.

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

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

S419

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

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Background: chronic myelomonocytic leukemia (CMML) is characterized by increased proliferation and myelomonocytic lineage commitment of hematopoietic stem cells (HSCs). Mutations in the RAS-signaling cascade are frequently present in CMML patients and lead to a CMML-like myeloproliferative disorder (CMML-MPD) in mice via causing hyperplasia to GM-CSF. Loss of RAF kinase inhibitor protein (RKIP), a negative regulator of RAS signaling, is frequent in myelomonocytic and monocytic subtypes of acute myeloid leukemia (AML) and is often associated with RAS mutations. Moreover, RKIP loss has recently been shown to increase the proliferation of AML cell lines.

Aims: In this work, we aimed at investigating the role of RKIP in the development of CMML.

Methods: RKIP expression was measured by immunoblot and quantitative real-time PCR in 23 primary CMML patient samples as well as in CD34+ HSCs, B-lymphocytes, granulocytes and monocytes of four healthy donors. Sequence analysis of CMML samples was done with an Ion Torrent Next Generation Sequencing platform using an amplicon panel covering 39 genes recurrently mutated in myeloid neoplasms. Effects of RKIP on GM-CSF-induced myelomonocytic differentiation were studied in human CD34+ HSCs lentivirally transduced with RKIP shRNA, as well as in a genetic mouse model for RKIP deletion (RKIP-/-). Effects of RKIP on CMML development were initially studied in the same RKIP-/- model. Additionally, these mice were crossed with animals exhibiting a somatically inducible mutation in NRAS (RKIP-/-Mx1-Cre-NRASG12D) and the severity of CMML-MPD onset was studied at an age of six months.

Results: Loss of RKIP protein expression was observed in 6/23 (26%) CMML patient specimens and was associated with decreased mRNA levels as well (P<0.001). Patients with RKIP loss exhibited an increased percentage of myelomonocytic cells in the peripheral blood (56% vs 75%, P=0.0226). One or more mutations affecting the RAS signaling pathway were detected in all specimens with RKIP loss. In addition to the previously demonstrated induction of proliferation, we then aimed to delineate a role of RKIP loss in myeloid lineage commitment. When studying healthy donors, we observed that RKIP expression was high in HSCs and lymphoid cells, but significantly decreased in cells belonging to the myeloid lineage (monocytes, P=0.001 and granulocytes, P<0.001). In functional experiments, knockdown of RKIP increased the GM-CSF-induced myelomonocytic lineage commitment of both, human and murine HSCs (P=0.05 and P=0.0295, respectively). These results could be corroborated in vivo, as intraperitoneal injection of GM-CSF caused a significant increase of myelomonocytic cells in the intraperitoneal cavity (P=0.006, bone marrow (P=0.007) and peripheral blood (P=0.027) in RKIP-/- mice when compared to their wildtype littermates. In a final step, we evaluated the potential of RKIP loss to cause CMML-MPD in mice. While it proved to be insufficient to cause the disease as a single event in RKIP-/- mice, it aggravated the CMML-MPD phenotype in animals with a somatically inducible mutation in NRAS (RKIP-/-Mx1-Cre-NRASG12D) and the severity of CMML-MPD onset was studied at an age of six months.

Summary/Conclusions: RKIP loss is a frequent event in CMML and is associated with mutations affecting the RAS signaling cascade. Loss of RKIP is functionally involved in myelomonocytic lineage commitment of HSCs and aggravates CMML-MPD development in mice carrying an additional mutation in NRAS.

S420 JAK2 V617F HAEMATOPOIETIC CLONES WITH DIFFERENT EXPANSION KINETICS ARE DETECTABLE SEVERAL YEARS PRIOR TO MPN DIAGNOSIS

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Background: JAK2 V617F is the most common somatic mutation in the classical myeloproliferative neoplasms (MPNs) and is also frequent amongst healthy individuals with age-related clonal haemopoiesis (ARCH).

Aims: To investigate the pre-clinical clonal evolution of MPNs.

Methods: We identified 12 individuals with JAK2 V617F mutant MPN from whom blood data was available from the time of MPN diagnosis and also from an earlier time point of between 4.5-15.2 years previously (median 10.2 years) when blood was donated for registration to the Cyprus Bone Marrow Donor Registry. We used deep DNA sequencing to interrogate all 24 samples at 15 myeloid mutation hotspots including JAK2 V617F, using an established multiplex PCR/MiSeq sequencing protocol that reliably detects nucleotide substitutions present at a variant allele fraction (VAF) ≤0.008. Additionally, for 12 samples with sufficient DNA available, we performed targeted DNA capture for all exons of 41 genes recurrently mutated in myeloid neoplasms using a custom RNA-bait library followed by sequencing on Illumina HiSeq 2500. Finally, we genotyped archived Registry samples for the rs12343887 single nucleotide polymorphism (SNP) (G/T) linked to the JAK2 46/1 haplotype.

Results: Amplicon sequencing returned a median coverage of 6641 reads per nucleotide (nt) at the studied hotspots. This confirmed the presence of JAK2 V617F in all 12 diagnostic and 9 of 12 archival samples. The remaining 3 samples were JAK2 V617F negative at the sensitivity of our assay (VAF≤0.008). The only other hotspot mutation identified was SRSF2 P95R in one patient, P3, whom had a diagnosis of myelofibrosis. Pulldown sequencing of all exons of 41 genes from 12 samples with sufficient DNA returned an average coverage of 1978 reads per nt and showed a close correlation in JAK2 V617F and SRSF2 P95R VAF quantitations with amplicon sequencing. The JAK2 V617F VAF at the diagnostic time point differed between patients as expected whereas the average rate of clonal growth also varied widely between individuals, ranging from 0.36 to 6.2% per annum (Figure 1). Targeted exon capture from 12 of 24 samples, only identified one co-mutation with a VAF >0.02, the SRSF2 P95R in patient P3. As this locus was also amplified by amplicon sequencing, we were able to quantify the SRSF2 P95R VAF in both the diagnostic and the archival DNA sample taken 12.6 years earlier. In the P3 diagnostic sample the VAFs for JAK2 V617F and SRSF2 P95R were similar (0.37 and 0.41 respectively) indicating that they co-occurred in most cells of the neoplastic clone. In the archival sample from P3, the SRSF2 P95R was detectable at a VAF of 0.06, however the JAK2 V617F was absent/undetectable at the sensitivity of our assay (VAF≤0.008) indicating the SRSF2 P95R was the clone-founding mutation in this neoplasm. The genotyping results for the rs12343887 SNP revealed a tentative association in our small cohort between homozygosity for the risk allele (C) linked to the JAK2 46/1 haplotype and the average annual increase in JAK2 V617F VAF. This will need to be verified in larger studies.

Summary/Conclusions: Our findings reveal that JAK2 V617F neoplasms develop from clonal haematopoiesis over many years. The rate of clonal expansion of JAK2 V617F clones in the pre-clinical phase was highly variable and although it was tentatively associated with the 46/1 haplotype, the high variability observed suggests that other factors likely influence clonal progression.

S421 DISRUPTION OF HAEMATOPOIETIC STEM CELL HETEROGENEITY IN A MOUSE MODEL OF MYELOPROLIFERATIVE NEOPLASM

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Figure 1.
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Background: The hematopoietic stem cell (HSC) compartment in mice encompasses a broad range of heterogeneous cell types including highly lineage-biased HSCs, such as platelet-biased HSCs (PMID:23934107). Myeloproliferative neoplasms (MPNs) are a heterogeneous spectrum of clonal hematopoietic disorders, that includes essential thrombocytosis (ET), a MPN-subtype usually presenting with isolated thrombocytosis. Most ET patients carry a gain-of-function point mutation in JAK2 (JAK2V617F), with several other collaborating hits reported to co-occur with JAK2V617F at lower frequencies, including abnormal gene expression in EZH2, which are more frequent in advanced MPN.

Aims: Although it is broadly accepted that MPNs are propagated by counterparts of HSCs, the impact of collaborating MPN-associated mutations arising in different HSC subsets remains unclear. We aimed to explore the possibility that platelet-biased HSCs might selectively promote development of an ET phenotype.

Methods: We generated a novel mouse model of MPN that carries a conditional knock-in of heterozygous human JAK2V617F (hJAK2V617F) and the conditional knock-out (KO) of EZH2 together with an inducible Mx1-Cre transgene. To analyse platelet-biased HSC subsets upon onset of the mutation(s), we also crossed in the vwf-eGFP transgene, which is selectively expressed in parts of HSCs, the impact of collaborating MPN-associated mutations arising in different HSC subsets remains unclear. We aimed to explore the possibility that platelet-biased HSCs might selectively promote development of an ET phenotype.

Results: Compared to wild-type and single mutant mice, EZH2-KO hJAK2V617F mice showed increased platelet counts, including a subset of mice which became acutely unwell with an extreme thrombocytosis. Strikingly, in serial bone marrow (BM) transplantation assays, EZH2-KO fully rescued the previously described hJAK2V617F-associated transplantation defect (PMID:20489053). EZH2-KO hJAK2V617F BM recipients showed long-term serial engraftment that was fully restricted to the platelet and myeloid lineages with a persistent thrombocytosis and absence of lymphoid reconstitution. RNA-sequencing revealed upregulation of several signaling pathways, including Hedgehog, and increased inflammation associated gene expression in EZH2-KO hJAK2V617F HSCs. Unexpectedly in this mouse model of thrombocytosis, phenotypic analysis of the HSC compartment in the BM showed that vwf-eGFP+ve HSCs were selectively lost (fold change[FC]=0.12 p=0.009), while vwf-eGFP–ve HSCs from Ezh2-KO hJAK2V617F mice primarily gave rise to platelet-biased HSCs. In contrast, vwf-eGFP+ve HSCs from Ezh2-KO hJAK2V617F mice engrafted poorly without recapitulating the disease in recipients.

Summary/Conclusions: In this novel Ezh2-KO hJAK2V617F mouse model, EZH2 loss collaborates to worsen thrombocytosis and rescue the HSC function defect in hJAK2V617F mice. We also observed a striking disruption of the germinal and functional HSC heterogeneity in Ezh2-KO hJAK2V617F mice with an unexpected and selective loss of vwf-eGFP+ve HSCs 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 adequate efficacy based on the findings from a bioequivalence study in adults. Primary objectives were major cytogenetic response (MCyR) for CML-CP resistant/intolerant to IM and complete cytogenetic response (CCyR) for newly diagnosed CML-CP (MCyR >30% and CCyR >55% considered of clinical interest). Study cohorts were not designed to be comparative.

Results: From 145 pts enrolled, 130 were treated; 54% were aged ≥12-<18 years. Within the IM-resistant/intolerant group, 25 were resistant, 2 were intolerant, and 2 were undetermined. For pts with CML-CP (n=113), 48% of pts with IM-resistant/intolerant CML-CP and 73% with newly diagnosed CML-CP remained on treatment at the time of this analysis (table 1). Cumulative rate of MCyR for IM-resistant/intolerant CML-CP remained as 3 months for IM-resistant/intolerant CML-CP, and a cumulative rate of CCyR >55% was reached as early as 6 months for newly diagnosed CML-CP (table). Estimated progression-free survival (PFS) by 48 months was 78% for IM-resistant/intolerant CML-CP and 93% for newly diagnosed CML-CP (table). Reasons for progression were loss of MCyR (n=3 IM-resistant/intolerant; n=4 newly diagnosed), loss of complete hematologic response (n=2 each), and development of CML-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-induced neutropenia, fever, or adenaemia was observed. In addition, no pulmonary arterial hypertension were reported here. Heterosensitivity 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 that DAS were met as early as 3 and 6 months, respectively, and deep responses were observed. Efficacy and safety of DAS in pediatric pts were similar to those observed in adults; however, unlike in adults, no cases of pleural/pericardial effusion were reported.}

Figure 1. Summary/Conclusions: The present 24 month RFS of 77% for the overall 24 months in patients in stable MR4 appears better than in any comparable study to date, and implies that the initial 12 months of dose reduction may be respon-

possible, perhaps via improved compliance in the few months prior to stopping or through an as yet undefined mechanism.

S424

ASSESSMENT OF IMATINIB 400MG AS FIRST LINE TREATMENT OF CHRONIC MYELOID LEUKAEMIA: 10-YEAR SURVIVAL RESULTS OF THE HUMANIZED CML STUDY IV


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Figure 1.
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Background: The optimum initial treatment of chronic myeloid leukemia (CML) is unknown.

Aims: CML-study IV was designed to confirm the International Randomized Study on Interferon (IFN) and STI571 (IRIS) and to explore whether treatment with imatinib (IM) at 400mg/day could be optimized.

Methods: From July 2002 to March 2012, 1551 newly diagnosed patients in chronic phase (CP) were randomized into a 5-arm study. 1536 patients were evaluable, 400 for IM400mg, 430 for IM + IFN, 420 for IM800mg, 158 for IM + cytarabine and 128 for IM-after-IFN-failure. Recruitment to the latter two arms was stopped after a pilot-phase.

Results: After a median observation time of 9.5 years, 10-year overall survival (OS) of all patients was 82%, 10-year progression free survival 80% and, 10-year relative survival 92%. 10-year OS was 80% with IMATINIB FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA: INITIAL RESULTS FROM THE BFORE TRIAL

Monotherapy with IM400mg provides a close to nominal survival. Outcome of CML is currently more determined by disease biology and demographics than by treatment optimization.

S425

BOSUTINIB VS IMATINIB FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA: INITIAL RESULTS FROM THE BFORE TRIAL

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Background: Bosutinib (BOS) is a potent, dual SRC/ABL tyrosine kinase inhibitor approved for treatment of adults with Philadelphia chromosome-posit

P<0.02). Rate of complete cytogenetic response (CCyR) by 12 months was also significantly higher with BOS versus IM (77.2% vs 66.4%; P=0.038), with time to CCyR shorter for BOS (hazard ratio=1.38; P<0.001). Rate of BCR-ABL transcripts ≤10% (Intl. Scale) at 3 months was higher with BOS versus IM (75.2% vs 57.3%; P=0.001); rates of deep molecular response over time were also generally higher with BOS (Table). Results for molecular endpoints were similar in the mITT 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 (7.8% vs 1.1%) 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% 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. Conclusions 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.
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 molecular remission and in major molecular remission (3-log reduction in BCR-ABL l1 levels) (Mahon ASH 2016). Between patients 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 1 detectable ABL1 copy) compared to the MR4 (at least 1 detectable ABL1 copy) in EURO-SKI (Pfeiffer ASH 2016). Aims: For 91 of 448 patients of the EURO-SKI learning sample, the sensitivity to investigate undetectable disease at the MR4.5 level was not given. Aim was to determine whether RFS probability 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 not MR4.5 detectable) and “MR4 undetectable disease” (MR4 without detectable BCR-ABL1), patients were stratified according to the number of control gene transcripts. For which small differences in GUSB 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 for MR4 detectable disease (78,975 vs 68,925 with undetectable disease; P=0.05, not significant (n.s.)). In 196 patients with detectable disease, RFS at 6 months was 52% (95% confidence interval (CI): 45-59%), patients with undetectable disease 63% (95% CI: 53-73%) (P<0.01). Relapse was significantly higher in patients with detectable disease (odds ratio: 1.630: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% RFS probability and patients with undetectable disease 59% (CI: 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: 0.862-1.984, n.s.).

Summary: By adjusting for 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.

AML Biology II: Epigenetic targets

S427

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

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Background: Deregulated expression programs due to genetic alterations, such as gene fusions affecting transcription and/or epigenetic factors is the hallmark of acute myeloid leukemia (AML) and the basis for the associated differentiation block of hematopoietic progenitors. Acute megakaryoblastic leukemia (AMKL) is a subtype of AML affecting primarily young children. Recently, the ETO2-GLIS2 fusion has been identified in 20-30% of de novo AMKL and associated with the worst prognosis in this subtype of AML.

Aims: Our goal was to characterize the mechanisms of cellular transformation induced by ETO2-GLIS2.

Methods: We first defined the consequences of ETO2-GLIS2 expression on hematopoietic progenitors and the contribution of ETO2 and GLIS2 on differentiation and self-renewal by methylcellulose replating assays and phenotype characterization. We then assessed global expression profiling and ETO2-GLIS2 direct binding on DNA by ChIPseq experiments. With immunoprecipitation experiments, we identified some ETO2-GLIS2 complex members. Finally, we tested the effects of a small peptide that could inhibit ETO2-GLIS2 complex stabilization both in vitro and in vivo.

Results: We showed that the GLIS2 moiety drives the megakaryocytic phenotype whereas both the ETO2 and GLIS2 moieties are required for maintaining self-renewal. Global expression profiling and comparison to patients’ signature consistently identified ETO2-GLIS2-mediated deregulation of major transcriptional regulators of hematopoiesis and leukemogenesis. Especially, ETO2-GLIS2 brings on an imbalance in ETS/GATA factors illustrated by an extinction of GATA1 and an overexpression of the ERG oncogene. We identified that ETO2-GLIS2 complex directly binds DNA via ETO2 complexes and through its GLIS2 moiety. Moreover, the ETO2-GLIS2 fusion localizes at half of H3K27ac-enhancers, so called super-enhancers, to control transcription of associated genes, in close association with ERG. Dimerization of ETO2-GLIS2 and interaction with endogenous ETO2 via its NRHR2 domains were demonstrated with in vivo recombination experiments. The ETO2-GLIS2 complex inhibited the oligomerization, reversed the transcriptional activation at enhancers, promoted megakaryocytic differentiation and abrogated human AMKL cells maintenance in vivo. So, the interaction of ETO2-GLIS2 with ETO2 complexes is an essential node for the transcriptional control by the fusion at enhancer elements. Finally, ETO2 is localized at super-enhancers and is recruited upon up-regulation of associated genes. ERG knockdown or genetic inactivation downregulates expression of ETO2-GLIS2 targets required for leukemic cells survival. Together, the strong up-regulation of ERG by the fusion and the presence of ERG at super-enhancers suggest a feed forward mechanism to impose gene deregulation.

Summary/Conclusions: In conclusion, we propose that the megakaryocytic differentiation arrest and self-renewal controlled by ETO2-GLIS2 results from imbalanced expression of master transcription factors imposed by aberrant chromatin structures at enhancers that may be disrupted by targeting the NRHR2 interface.

S428

NUCLEOSOME BINDING PROTEIN HMGN1 BLOCKS MYELOIDDIFFERENTIATION AND PROMOTES CLONAL DOMINANCE VIA ABBERRANT HISTONE H3 ACETYLATION

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Background: Acute myeloid leukemia (AML) is characterized by rapid growth and block in differentiation of myeloid progenitors. The AML blast is defined by having “open” chromatin. We hypothesized that alterations of chromatin conformation may promote AML. Reversing those changes could represent a novel therapeutic approach.

Aims: Gain of chr21q22 is the most common focal amplification in complex karyotype AML. HMGN1 is a chromatin-regulatory protein on 21q22 known to affect lymphoid development, and our preliminary data suggested that HMGN1 could directly mediate a myeloid differentiation block. Since HMGN1 is known to decompact chromatin and alter histone marks, our goal was to define and therapeutically target the mechanisms by which HMGN1 overexpression disrupts myeloid differentiation and promotes clonal dominance.

Methods: We immortalized bone marrow progenitors from wild-type (WT) or OE-HMGN1 mice (transgenic overexpressing HMGN1) with an estrogen receptor-HoxB8 fusion protein. Using exogenous estrogen to control translocation of HoxB8, we analyzed synchronized myeloid differentiation by flow cytometry, RNAseq, and TMT proteomic analysis. We performed MINT-Chip-seq (MNase Indexed T7-chromatin IP) to measure the histone marks H3K27ac, H3K27me3, H3K4me3 and total Histone H3. We also measured histone marks in hematopoietic stem and progenitor subpopulations in vivo. We performed competitive bone marrow transplantation with CD45.1 WT and CD45.2 OE-HMGN1 donors and measured the relative contribution to hematopoiesis over time.

Results: Synchronized differentiation in WT cells progressed over 6 days from myeloid progenitors to mature neutrophils and monocytes, analyzed by cell surface markers, morphology, and gene and protein expression. OE-HMGN1 cells proliferated faster and remained as undifferentiated myeloblasts (84% CD11b+Gr1+ in WT vs 4% in OE-HMGN1, p<0.002; Fig A). Gene set enrichment analysis revealed more similarity to undifferentiated hematopoiesis and leukemia signatures in OE-HMGN1 cells. MINT-Chip indicated a global and locus-specific increase of H3K27ac in OE-HMGN1 cells (Fig B, upper panel), consistent with an increase in gene transcription, confirmed by RNA-seq. We found a specific increase in HoxA cluster expression in OE-HMGN1 cells, consistent with an increase in gene transcription, confirmed by RNA-seq. We found a specific increase in HoxA clusters expression in OE-HMGN1 cells. High expression of PIWIL4 was associated with immature myeloid phenotype independent of HMGN1 expression. Ablation of PIWIL4 expression in AML cell lines revealed a global reduction in repressive H3K9me3 marks at promoters and gene body.

Conclusion: Our study suggests that HMGN1 overexpression blocks myeloid differentiation and promotes proliferation in hematopoietic progenitors via increased H3K27 acetylation. Targeting epigenetic changes downstream of HMGN1 or interfering with HMGN1 itself may represent a novel therapeutic strategy in AML.
Acquired and inherited platelet disorders

S431
THE COMBINATION OF ORAL ALL-TRANS RETINOIC ACID AND DANAZOL VS DANAZOL AS SECOND-LINE TREATMENT IN ADULT IMMUNE THROMBOCYTOPENIA: A MULTICENTRE, RANDOMIZED, OPEN-LABEL TRIAL

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by increased platelet destruction and impaired platelet production. Despite decades of basic and clinical research, the treatment of severe, corticosteroid-resistant or relapsed disease remains a great challenge. Our preliminary study indicated the effectiveness of all-trans retinoic acid (ATRA) for ITP (Wang M, et al. ASH 2012, Abstract #3338). This has been coupled with previous discoveries of an immune-modulation effect of ATRA in ITP, including its role to induce changes in Treg cells (Ruan CG 2016), and to correct the imbalance of aberrant macrophage polarization (unpublished data), indicating ATRA as a potential therapeutic regimen. Danazol has been used in the treatment of ITP for more than 30 years. Apart from its haemopoietic stimulatory and immune-modulatory effect, it has recently been shown to reverse abnormal thrombopoiesis in patients with thrombocytopenia (Townsley DM et al. 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.

S432
NOVEL PERSPECTIVES IN GENOTYPE-PHENOTYPE CORRELATIONS IN MYH9-RELATED DISEASE: NO LONGER JUST A MATTER OF HEAD OR TAIL

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Background: MYH9-related disease (MYH9-RD) is an autosomal-dominant disorder caused by mutations in MYH9, the gene for non-muscle myosin heavy...
chain II (NMHC-IIA), and represents the most frequent inherited thrombocytopenia worldwide. NMHC-IIA comprises two distinct domains, the N-terminal globular head domain (HD) and the C-terminal tail domain (TD), and causative mutations hit either the HD or the TD. All patients present at birth with macrothrombocytopenia and only some of them develop during life additional manifestations, including nephropathy often leading to end-stage renal disease (ESRD), sensorineural deafness, and/or cataract. Thus, the search for genotype-phenotype correlations in MYH9-RD has been an important research topic since the identification of the disorder. In 2008, the analysis of 108 patients allowed to conclude that the mutations affecting the HD were associated with evolution to early-onset ESRD and deafness, whereas the risk of manifesting HD-related manifestations was much lower for patients carrying mutations of the TD. In 2014, raising to 255 the number of patients, we suggested that evolution to juvenile ESRD associated only with the most frequent among HD mutations, i.e. substitution of the arginine 702 (R702). Conversely, the TD-related mutations were almost exclusively carried in a distal region of the HD protein, i.e. in the C-terminal region at the interface between the SH3 subdomain and the motor domain (SH3/MD interface), may be associated with a much less severe evolution.

Aims: To improve prognostic assessment of patients with MYH9-RD.

Methods: All the consecutive patients enrolled in the Italian registry for MYH9-RD until June 2015 were included. The association of MYH9 genotype with phenotype was assessed by a generalized linear regression model (event-free survival analysis).

Results: We enrolled 350 patients belonging to 199 MYH9-RD pedigrees. Mutational screening allowed us to identify 6 novel causative mutations in the HD of 6 different pedigrees. Interestingly, all of these variants were localized in the hydrophobic region of the SH3/MD interface. By raising the number of patients with mutations in this region from 14 to 26, and increasing the observation time, we could demonstrate that the mutations in the SH3/MD interface are associated with an earlier development of deafness at young age, but low risk of kidney disease and cataract. The other previously identified genotype-phenotype correlations were confirmed. In particular, mutations hitting the R702 in the HD resulted in constant evolution toward juvenile ESRD and severe deafness. Among mutations different from R702 substitutions, the p.D1424H in the TD associated with the highest risk to develop non-congenital manifestations of the disease.

Summary/Conclusions: Mutations in the HD of the NMHC-IIA are almost all localized in a specific region at the SH3/MD interface, which therefore represents a critical region for MYH9-RD pathogenesis. Most importantly, patients with mutations of this HD variant were distinguished into two different groups: subjects with R702 substitutions are expected to develop early severe a syndromic disorder, whereas mutations in the SH3/MD interface are associated with evolution to a milder phenotype, characterized by development of hearing impairment only (“auditory” phenotype). Our study confirmed a genotype-phenotype model for MYH9-RD that overcomes the previously reported dualism between HD vs TD mutation.

S433

A MONOALLELIC LOSS-OF-FUNCTION MUTATION IN THE THROMBOPOIETIN (THPO) GENE IS RESPONSIBLE FOR A NEW FORM OF INHERITED THROMBOCYTOPENIA

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Background: The THPO-MPL 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 to result from abnormal MK functioning: congenital thrombocytopenia, 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 thrombocytopoiesis (ITP) present at birth with isolated thrombocytopenia, which always evolves into severe bone marrow aplasia. Similarly, a homozygous loss-of-function variant in the 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 Working Group on alpha-granule abnormalities. The identification of these 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 proposals with unknown IT identified 2 unrelated individuals (family A and B) carrying the heterozygous variant c.377C>T (p.Arg13Cys) 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 MK morphological abnormality. Interestingly, a few nuclear elements were slightly increased in sizes 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 thrombocytopoenia and the absence of qualitative platelet defects, at least in the two patients 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 p.Arg317 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 ETβ6, ANKR2D6 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 GF1B ZINC FINGER MUTATION DECOUPLES CD34 EXPRESSION FROM ALPHA-GRANULE DEFICIENCY IN GF1B-RELATED PLATELET DISORDERS

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Background: GF1B is a transcription factor that plays an important role in haematopoiesis. Families with a mutation of the fifth DNA-binding zinc-finger domain of GF1B experience bleeding and have a platelet phenotype characterised by macrothrombocytopenia, increased CD34 expression and alpha-granule deficiency. Aims: To explore the function of other zinc finger domains of GF1B we have characterised two unrelated families with a GF1B variant, C168F, predicted to disrupt the first zinc-finger domain and compared the phenotype with a previously described pedigree with the H294fs mutation that disrupts the fourth zinc-finger domain.

Methods: Clinical platelet phenotypes were determined by light and transmission electron microscopy and functional studies performed by light transmission and whole blood impedance aggregometry. Platelet protein expression was measured by flow cytometry and western blotting. DNA-binding of variants was determined by gel mobility shift assays (EMSA) and changes in gene transcription by luciferase assays. Cellular phenotypes were then studied in patient specific IPSCs derived megakaryocytes.

Results: Individuals with both C168F and H294fs are thrombocytopenic (mean platelet count =107 x109/L, n=8) but lack the collagen induced aggregation defects and bleeding symptoms observed in individuals with H294fs (ISTH BAT, P=0.015). Alpha granule content observed by microscopy and quantitated by western blotting of granule related proteins, P-selectin and fibrinogen, were similar between C168F and control platelets and this was significantly greater than that observed for the H294fs mutation (P<0.01). EMSA studies indicate that the C168F variant retains the ability to bind DNA whereas the H294fs mutation altering Zn finger 5 abrogates DNA binding. Despite retaining the ability to bind DNA, the C168F variant de-represses gene transcription at TUBB1, CYR61 and TUBB2 (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 blotting of CD34 with both variants (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 blotting of CD34 with both variants (P<0.01). The transcriptional de-repression observed at the CD34 promoter 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 GF1B zinc finger 1 cause thrombocytopenia with increased CD34 expression but these platelets retain relevant clinical bleeding symptoms. This finding is mechanistically distinct from the Zn finger 5 mutation that abrogates DNA binding with a subsequent phenotype characterised by alpha-granule deficiency and clinical bleeding.
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: CITP is characterized by autoantibody-directed platelet destruction mediated by activated monocyte Fc receptors which signal via spleen tyrosine kinase (syk). A Phase 2 trial of the oral syk inhibitor Fostamatinib (FOSTA) in 16 patients (pts) with refractory CITP 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. Sixty-one % of pts were female; median age was 54 y (20-88); 93% were Caucasian; and stratification by prior splenectomy and baseline plt ct.<or ≥15K/µL. Sixty-three (S047, 74 in S048) of 24 weeks duration, followed by an open label study (S049).

Results: Across both studies, a SR occurred in 18/101 (18%) FOSTA vs 1/49 (2%) placebo pts; but not in the 29 responders. The number of pts with ≥1 adverse event (AE) was comparable 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 ct in certain pts with heavily pre-treated, severe citP of long disease duration. AEs are mostly mild or moderate in severity. Given its unique mechanism of action-based on inhibition of syk, FOSTA could, if approved, be an important alternative as single agent and be a useful component of combination therapy for pts with difficult citP.

References

Acute lymphoblastic leukemia - Biology

S436

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


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

Aims: Understanding the pathogenesis of relapse of DS-ALL relating to their CRLF2 status.

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

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

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TNF RECEPTOR 2 IS REQUIRED FOR RIP1-DEPENDENT CELL DEATH IN LEUKEMIA

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Background: Persistence of residual leukemia cells, due to deficiencies in apoptotic programs, is a major driver of relapse. Activation of alternative non-apoptotic cell death pathways such as necroptosis represents an attractive strategy to eliminate residual leukemia cells and prevent relapse. We have previously shown that SMAC-mimetics (SM) potently induce cell death by simultaneous RIP1-dependent apoptosis and necroptosis in a subset of refractory acute lymphoblastic leukemia (B-ALL) patient-derived samples. The molecular signals that drive sensitivity to RIP1-dependent cell death remained elusive so far.

Aims: The aim of this project was to understand the mechanisms that determine the specific vulnerability to necroptosis in ALL.

Methods: To identify molecular determinants of sensitivity to SM, we correlated the gene expression profiles of 17 primary samples with high and low sensitivity to SM with the IC50 in response to two SM compounds, birinapant and LCL161. We confirmed the top scoring genes including TNF receptor 1 (TNFR1) and TNFR2 by quantitative RT-PCR in patient-derived xenografts. We further validated our results by quantifying the expression of the candidate genes in an independent cohort of relapsed primary B-ALL and by screening samples with different expression levels of TNFR1 and 2 for their response to SM in vitro.

To assess the mechanistic role of TNFR1 and 2 in the response to SM, we generated patient-derived TNFR1 and TNFR2 knockout cells using CRISPR/Cas9 gene editing technology, and evaluated their response to SM in vitro and in vivo using a CRISPR selection model. Additionally, we overexpressed TNFR2 and evaluated the cell death phenotype. To determine the mechanism of TNFR1-mediated sensitization to SM, we investigated the formation of the pro-death RIP1-TNFR1 complex in wild type versus TNFR2 knockout cells and in SM sensitive and resistant ALL by immunoprecipitation in primary ALL samples.

Results: Comparative gene expression profiling indicated a correlation of the expression of TNFR2 with sensitivity to SM in primary ALL. Using an independent cohort of relapsed ALL samples, we found that high TNFR2 expression predicted sensitivity to SM in an ex vivo model of the bone marrow. Deletion of either TNFR1 or TNFR2 using CRISPR/Cas9 in patient-derived ALL conferred resistance to treatment with SM in vivo in the xenograft model, indicating that TNFR1 and 2 are both functionally required for cell death. In agreement with an important role for TNFR2 in the response to SM, the overexpression of TNFR2 leads to increased sensitivity to the TNFR1/RIP1 death axis. On the mechanistic level, recruitment of RIP1 to TNFR1 is a key event in the activation of cell death, which is abolished in TNFR2-deficient leukemia and does not occur in SM resistant cases.

Summary/Conclusions: Taken together, our data reveal a novel function of TNFR2 in cell death signaling, as TNFR2 predicts sensitivity to SMAC mimetics and plays a key role in activating the TNFR1/RIP1 cell death pathway, which underlies the switch from RIP1-controlled cell survival to cell death and characterizes a distinct vulnerability in ALL.

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 by 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-iCreertms2 R26-Mybhtg/tg mice developed T-cell lymphomas with a median latency of 77 weeks, suggesting that Myb can act as a bona fide oncogene in malignant T-cell transformation (Figure 1A). Next, we crossed our Myb transgenic model with Pten conditional knockout mice, to allow comparative analysis of tumors with and without T-cell specific Myb expression. Genetic inactivation of Pten is frequently observed in human T-ALL, and T-cell specific deletion of Pten (using Lck-Cre) results in T-cell leukemia/lymphoma development with an average of 17 weeks. Using this strategy, we obtained mice that overexpress R26-driven cMyb and lack Pten in developing T-cells and found that cMyb expression synergizes with Pten deletion, resulting in fully penetrant and accelerated T-ALL formation (median survival of 84 days instead of 118; p = 0.0003; Figure 1B). Finally, we used this novel murine T-ALL model to identify new therapeutic strategies for MYB dependent T-ALL. Importantly, the tumor cells from the cMyb knockin mice are luciferase-positive and are therefore suitable for in vivo drug testing using bioluminescence. Using this model, we evaluated the in vivo anti-leukemic efficacy of a variety of small molecules and identified new drugs that impede Myb protein stability or Myb-mediated transactivation in Myb driven tumorigenesis.
THE T-CELL LEUKEMIA ASSOCIATED RIBOSOMAL R10L 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 BaF3 cells expressing RPL10 WT or RPL10 R98S. Differences in protein expression were further validated in hematopoietic cells derived from a transgenic RPL10 R98S knock-in mouse model and in material derived from xenografted T-ALL patient samples.

Results: The differential proteome screen revealed overexpression of several Jak-Stat signaling components (Csf2rb2/2, 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 pimozone. RPL10 R98S positive leukemia patients likewise showed overexpression of IL7RA, Jak1 and Stat5, increased sensitivity to pimozone, 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 catalytinc 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.

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NFATC3-PLA2G15 IS A NOVEL INTERGENICALLY SPLICED CHIMERA THAT IS ASSOCIATED WITH AGGRESSIVE T-ACUTE LYMPHOBLASTIC LEUKAEMIA BIOLOGY

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

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

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

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

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

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

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**Background:** Cancer patients have a higher risk of venous thromboembolism (VTE) which conveys a higher subsequent mortality risk; conversely, they also have a higher risk for bleeding due to many factors including abnormal tumor anatomy and the use of chemotherapy agents with the associated risk for thrombocytopenia. However, the consequences of a recurrent VTE or a major bleeding event 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, we sought to estimate the risk and benefit of anticoagulant therapy in cancer patients developing a VTE using data from administrative databases.

**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 assessed within 180 days from the index date. MB was identified using a previously validated algorithm and included upper and lower gastrointestinal and intracranial bleeding events. Treatment was classified based on the first available prescription within 7 days of the index VTE. We estimated mortality within 7 days of the VTE recurrence or MB events using an unadjusted 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:** Lymphoma is a malignancy that originates in lymph nodes and lymphoid tissue. The main category of lymphomas is non-Hodgkin’s lymphoma (NHL). NHL comprise about 3% of all cancers in Sweden. Some are aggressive and fast growing, while others are more indolent and do not necessarily require treatment. It is well known that cancer increases the risk of thrombosis, especially in the case of thromboembolism, but data are scarce on the risk of thrombosis in NHL patients.

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

**Methods:** We conducted a nested case-control study consisting 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 attacks, angina pectoris, myocardial infarction, and arterial embolism and thrombosis.

**Results:** NHL patients had a statistically significant increase in risk of any type of thrombosis compared to controls (HR: 1.58, 95% CI: 1.53-1.62). The risk was significantly increased for all three types of thrombosis; deep vein thrombosis (HR: 1.71, 95% CI: 1.67-1.75), pulmonary embolism (HR: 1.31, 95% CI: 1.25-1.37) 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, independent of previous history of thrombosis (HR: 1.64; 95% CI: 1.59-1.69) no previous history, HR: 1.43; 95% CI: 1.37-1.50 if previous history of thrombosis). The incidence of thrombosis for NHL patients started to increase about five months before the diagnosis of NHL, and reached its peak 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.
and validation cohorts. The ThroLy model was developed using data solely from a derivation cohort, which included 1236 patients. Variables were evaluated by univariate logistic regression analysis, while the model was developed using a stepwise multivariate logistic regression analysis. Once a final model was defined, patients were divided into low risk and at risk groups. The final model was assessed in the validation cohort (584 patients). The studied population was also divided, based on Khorana and Padua score, into low risk and at risk groups.

Results: The study population included 1820 eligible lymphoma patients. The mean patient’s age was 53.1 years (range, 15–87 years). Most patients (83%) were newly diagnosed and had advanced stage disease: Ann Arbor stage III, 14.7% and stage IV, 44%. A total of 778 patients (42.7%) had high-grade lymphoma; 351 (19.3%) had low-grade lymphoma; 266 (14.6%) had HL; 156 (8.6%) had other forms; and 269 (14.8%) had CLL/SLL. Of all the patients included in the study, 99 (5.4%) developed at least one TE during the follow-up period. There were 73 patients with venous TE (73.7%), and 25 with arterial TE (25.3%), while 1 patient had both. Patients with aggressive NHL had significantly higher odds of developing TE compared to patients with any other lymphoma type (RR=1.5; 95% CI for RR 1.1–2.4; p=0.027). The incidence of thromboembolism was 81 (5.3%) in the newly diagnosed patients and 18 (6.2%) in relapsed patients. Overall, 35.4% (59/99) 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.

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**S444**

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

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Background: Oncologic-hematologic hospitalized patients constitute a group at high risk of venous thromboembolism (VTE). Current clinical practice guidelines recommend prophylaxis with low molecular weight heparin (LMWH) during hospitalisation, unless contraindicated. However, its underuse is a worldwide problem. Electronic alert systems (e-alerts) can improve the use of appropriate thromboprophylaxis and reduce the incidence of VTE.

Aims: To evaluate the impact of a new version (v2.0) of our e-alert system for VTE prophylaxis compared with the initial software version. Secondary endpoints try to identify predictive factors for prophylaxis use and thrombotic events.

Methods: Prospective study including consecutive adult cancer patients admitted at our centre. From April 2014 to June 2015 (first period) the initial e-alert system version remained operative and from July 2015 to December 2016 (second period) the new version was active. The v2.0 displayed a second window that asked physicians about the reason why LMWH was not prescribed. The main outcomes were: VTE events during hospitalisation and 30 days after discharge. Descriptive statistical analysis and correlation between clinical variables and main outcomes were performed by using the software package SSPS v20.

Results: 1251 patients were included, 782 patients in the first period and 469 in the second one (main clinical features are shown in Table 1). E-alerts v2.0 was associated with an increase of appropriate LMWH prophylaxis during hospitalisation (65.2% vs 72.2%, p=0.015). However, this improvement did not result in a reduction of VTE during admission or follow up (2.3% vs 2.3%; p=0.89). Interestingly, almost 80% of VTE events occurred despite LMWH use. No differences in the rate of major bleeding (2.8% vs 3.2%; p=0.83), and mortality (10.6% vs 14.3%; p=0.07) were observed, either. The main reason for not prescribing LMWH prophylaxis was bleeding risk, but in 17% of cases physicians did not consider that the patient really had a high VTE risk. No significant correlation was found between any of the clinical variables analyzed and the risk of VTE. Prophylaxis use was more frequent among patients with solid cancer (vs hematologic), advanced stage, active chemotherapy treatment and longer hospital stay.

Summary/Conclusions: The new e-alert system further increases the use of VTE prophylaxis in hospitalised cancer patients, although this was not associated with a reduction in the VTE incidence. A relevant number of VTE events occur despite prophylaxis with standard LMWH. Identification of risk factors for thromboprophylaxis failure is needed.

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**S445**

**IDENTIFICATION OF A NEW AND RELATIVELY FREQUENT SERPINC1 GENE DEFECT CAUSING ANTITHROMBIN DEFICIENCY HARDLY DETECTED BY CURRENT MOLECULAR METHODS: DUPLICATION OF EXON 6**

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Background: Antithrombin (AT) deficiency was the first thrombophilia described 50 years ago and so far the strongest one. Up to 78% of cases are explained by point mutations or small deletion/insertions in exons or flanking regions of SERPINC1 that are easily detected by sequencing analysis. A low proportion of cases (2%) is explained by gross gene defects, mainly deletions, which are detected by multiplex ligation-dependent probe amplification (MLPA) analysis. However, the molecular base of AT deficiency is unknown using current methods in 20% of cases.

Aims: To identify new SERPINC1 defects causing AT deficiency.

Methods: We studied 271 unrelated cases with AT deficiency. Functional and biochemical assays characterized plasma AT. Genetic analyses involved Sanger and Next Generation Sequencing (NGS) (PGM, Ion Torrent), MLPA and specific PCR designs.

Results: Sanger sequencing of PCR amplicons with primers flanking the 7 exons and further analysis with SeqscapeTM detected pathogenic mutations in 173 cases. Whole gene sequencing identified 5 mutations in regulatory regions. MLPA analysis revealed 5 cases with whole or partial deletion of the gene. Moreover, 13 cases had disorders of glycosylation. Interestingly, the analysis of the PCR product and the electropherogram of exon 6 of a 42-year-old male patient (P1) with deep venous thrombosis and 75% of anti-FXa activity

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Table 1. Clinical features in Group1, Group 2 and both.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Age (years)</th>
<th>Mean APTT (s)</th>
<th>Mean DD (μg/L)</th>
<th>Mean FDP (μg/L)</th>
<th>Mean AT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>65.8±1.5</td>
<td>16.5±1.5</td>
<td>63.2±1.5</td>
<td>22.5±1.5</td>
<td>66±1.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>67.0±1.5</td>
<td>17.0±1.5</td>
<td>64.0±1.5</td>
<td>23.0±1.5</td>
<td>67±1.5</td>
</tr>
<tr>
<td>Both</td>
<td>66.4±1.5</td>
<td>16.7±1.5</td>
<td>63.6±1.5</td>
<td>22.8±1.5</td>
<td>66.5±1.5</td>
</tr>
</tbody>
</table>

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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 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. 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.
**Aims:** The aim of the study is to investigate whether donor CD4 T cells recognizing mismatched HLA-DR 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-DR mismatched, donors with 0.25-0.50 x 10^6/kg donor CD4 T cells to promote immune reconstitution. In 4 patients, Graft-versus-Leukemia reactivity and/or organ specific GVHD occurred after the infusion. To characterize the immune responses in these patients, in vivo activated T cells were clonally isolated and tested for reactivity against a panel of target cells, including patient and donor derived hematopoietic cells, third party hematopoietic cells as well as different GVHD target cells (patient skin fibroblasts, third party colon carcinoma cells, biliary epithelial cells and lung fibroblasts) expressing the mismatched, patient variant, HLA-DR molecule.

**Results:** Allo-HLA-DR directed CD4 T cells showing differential recognition of target cells were found in all 4 patients. A total of 33 HLA-DPB1*04:01 reactive CD4 T cell clones were isolated from patient 1 who suffered GVHD of skin and colon, but not liver. Within these 33 clones, 3 clones recognized only hematopoietic target cells, 9 clones recognized hematopoietic, skin and colon derived target cells and 5 clones recognized hematopoietic and colon derived cells only. None of the T cell clones recognized biliary epithelial cells. From patient 2 total of 230 HLA-DPB1*03:01 reactive CD4 T cell clones were isolated, of which 27 recognized only hematopoietic target cells and 96 clones also recognized GVHD target cells with differences in tissue specificity. 32 HLA-DPB1*03:01 reactive CD4 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 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-DR 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-DR alleles can be used to mediate tumor specific immune responses after HLA 10/10 matched unrelated stem cell transplantation.

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**S449**

**ABERRANT T CELL RESPONSES IN THE BONE MARROW MICROENVIRONMENT OF PATIENTS WITH POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background:** Poor graft function (PGF) remains a life-threatening complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT), and the underlying mechanisms have not yet been elucidated. Considerable evidence from murine studies has demonstrated that effective hematopoiesis depends on the specific bone marrow (BM) microenvironment, where hematopoietic stem cells reside. In this regard, we previously reported that PGF patients had impaired BM endosteal and vascular microenvironment (BBMT 2013; BMT 2016; Oncotarget 2016; Blood 2016). Moreover, our pilot study showed that both CD4+ and CD8+ T cells polarized towards a type 1 immune response in the BM microenvironment of PGF patients (N=10) compared to those in matched good graft function (GGF) patients (N=20) (BBMT 2016). Nevertheless, whether abnormalities of T cell subsets in the BM immune microenvironment, including Th17, Th1, Th1, Th2, Tc2 cells and regulatory T cells (Tregs), are involved in the pathogenesis of PGF remains to be explored.

**Aims:** To compare the T cell subsets in the BM immune microenvironment, including Th1, Th2, Tc2, Th17, Tc17 cells and Tregs, between patients with PGF and GGF after allo-HSCT.

**Methods:** This prospective nested case-control study enrolled 20 patients with mismatched related or unrelated donors undergoing allo-HSCT and 20 healthy donors (HD). The percentages of Th1 (37% vs. 26.4%, P=0.0005) and Th17 (19% vs. 18.4%, P=0.0001) cells were significantly higher in PGF patients than in GGF patients, whereas the percentages of Th2 (0.8% vs. 2.4%, P=0.0001) and Tc2 (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.
Aberrant B-cell homeostasis has been described in patients (pts) with chronic graft-versus-host disease (cGVHD) following allologeneic stem cell transplantation (allo-SCT). However, there is no information on the predictive value of specific B-cell subsets of the incidence of cGVHD.

**Aims:** We sought to determine if B-cell subsets measured around day 100 after allo-SCT predict the subsequent occurrence of cGVHD in a prospective clinical study.

**Methods:** Peripheral blood (PB) samples were obtained from consented patients (pts) between day 80 and 110 (D100) after allo-SCT at The University of Texas MD Anderson Cancer Center from 2012 to 2015. Only pts who had not been diagnosed with cGVHD or progression of underlying malignancy by D100 were eligible for this study. We analyzed CD19+CD20+ B cell subsets by FACS. Subsets were defined as naïve (CD27-IgD+), unswitched (CD27-IgD-), 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 mycophenolate mofetil for GVHD prophylaxis. Diagnosis was myeloid (61%) or lymphoid (34%) malignancy in the majority of pts. Grafts source was primarily PB or bone marrow from matched-unrelated (61%) or related (24%) donors. Grade ≥3 malignancy in the majority of pts. Grafts source was primarily PB or bone marrow from matched-unrelated (61%) or related (24%) donors.

**Summary/Conclusions:** In conclusion, D100 frequency of naïve and switched B cells predicts the subsequent development of cGVHD. Lymphoid malignancies and older age may be associated with aberrant B-cell reconstitution. Consideration of D100 B-cell subsets may improve risk stratification models for the development of cGVHD.

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**Figure 1.**

**Summary/Conclusions:** In conclusion, D100 frequency of naïve and switched B cells predicts the subsequent development of cGVHD. Lymphoid malignancies and older age may be associated with aberrant B-cell reconstitution. Consideration of D100 B-cell subsets may improve risk stratification models for the development of cGVHD.

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**S451**

**EFFECTS OF AG-348, A PYRUVATE KINASE ACTIVATOR, IN PATIENTS WITH PYRUVATE KINASE DEFICIENCY: UPDATED RESULTS FROM THE DRIVE PK STUDY**

**Background:** Pyruvate kinase (PK) deficiency is a congenital hemolytic anemia caused by deficiency of the glycolytic enzyme, red cell PK (PK-R). AG-348 is an orally available, small molecule, allosteric activator of PK-R that activates the wild-type and a range of mutated PK-R enzymes in vitro, and increases PK-R activity and restores adenosine triphosphate levels in red blood cells from patients with PK deficiency.

**Aims:** To report on preliminary efficacy and safety data from the use of AG-348 in the ongoing DRIVE PK study (NCT02476916), an open-label dose-ranging trial of AG-348 in transfusion-independent adults with PK deficiency.

**Methods:** After providing informed consent, patients were randomized to AG-348 50mg or 300mg orally twice daily (BID) for 6 months (Core Period). At the end of the Core Period, patients can continue on treatment for another 2 years in the Extension Period. Transfusion independence is defined as ≤3 units of red blood cells transfused in the 12 months preceding the first dose of AG-348 and no transfusions in the 4 months preceding the first dose. Patients are followed weekly for Weeks 1-3, every 3 weeks for Weeks 13-24 and then every 3 months until the end of the study. Hormone and iron status are evaluated at Baseline, Week 12 and End of Core Period, and then every 6 months in the Extension Period.

**Results:** As of 18 Jan 2017, goal enrolment has been met and all 52 patients are evaluable for safety and efficacy; 24 have completed the Core Period and 23 are ongoing in the Core Period. Five patients discontinued from the Core Period, owing to adverse events (AEs) (n=2) or consent withdrawal (n=3). Of the 24 subjects who completed the Core Period, 21 entered the Extension Period and 20 are still on treatment; 1 was discontinued by the investigator. Patients are currently receiving doses ranging between <25mg BID and 300mg BID. Since the previous data cutoff date of 23 Sep 2016 (where N=34), AG-348 had been well tolerated, small molecule, allosteric activator of PK-R that activates the wild-type and a range of mutated PK-R enzymes in vitro, and increases PK-R activity and restores adenosine triphosphate levels in red blood cells from patients with PK deficiency.

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**Figure 1.**

**Summary/Conclusions:** In conclusion, D100 frequency of naïve and switched B cells predicts the subsequent development of cGVHD. Lymphoid malignancies and older age may be associated with aberrant B-cell reconstitution. Consideration of D100 B-cell subsets may improve risk stratification models for the development of cGVHD.

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**S452**

**STEM CELL TRANSPLANTATION IN PYRUVATE KINASE DEFICIENCY**

**Background:** PK-R deficiency is a congenital hemolytic anemia caused by deficiency of the glycolytic enzyme, red cell PK (PK-R). AG-348 is an orally available, small molecule, allosteric activator of PK-R that activates the wild-type and a range of mutated PK-R enzymes in vitro, and increases PK-R activity and restores adenosine triphosphate levels in red blood cells from patients with PK deficiency.

**Aims:** To report on preliminary efficacy and safety data from the use of AG-348 in the ongoing DRIVE PK study (NCT02476916), an open-label dose-ranging trial of AG-348 in transfusion-independent adults with PK deficiency.

**Methods:** After providing informed consent, patients were randomized to AG-348 50mg or 300mg orally twice daily (BID) for 6 months (Core Period). At the end of the Core Period, patients can continue on treatment for another 2 years in the Extension Period. Transfusion independence is defined as ≤3 units of red blood cells transfused in the 12 months preceding the first dose of AG-348 and no transfusions in the 4 months preceding the first dose. Patients are followed weekly for Weeks 1-3, every 3 weeks for Weeks 13-24 and then every 3 months until the end of the study. Hormone and iron status are evaluated at Baseline, Week 12 and End of Core Period, and then every 6 months in the Extension Period.

**Results:** As of 18 Jan 2017, goal enrolment has been met and all 52 patients are evaluable for safety and efficacy; 24 have completed the Core Period and 23 are ongoing in the Core Period. Five patients discontinued from the Core Period, owing to adverse events (AEs) (n=2) or consent withdrawal (n=3). Of the 24 subjects who completed the Core Period, 21 entered the Extension Period and 20 are still on treatment; 1 was discontinued by the investigator. Patients are currently receiving doses ranging between <25mg BID and 300mg BID. Since the previous data cutoff date of 23 Sep 2016 (where N=34), AG-348 had been well tolerated, small molecule, allosteric activator of PK-R that activates the wild-type and a range of mutated PK-R enzymes in vitro, and increases PK-R activity and restores adenosine triphosphate levels in red blood cells from patients with PK deficiency.

**Summary/Conclusions:** AG-348 is a novel, first-in-class PK-R activator undergoing clinical testing in patients with PK deficiency. The ongoing DRIVE PK study has now met goal enrolment of 52 patients, and data from these patients will be available at the time of presentation. Chronic daily dosing with AG-348 is well tolerated and has demonstrated clinically relevant, durable increases in hemoglobin (Hb) +1 g/dL. Hb responses were seen across a range of four doses, and were rapid and sustained. For a subset of patients (n=8), the rate of glycolytic metabolism in peripheral blood samples was assessed before and after treatment, and a positive correlation was observed between increases in glycolytic flux through the PK-R pathway and increases in Hb. Updates on safety, clinical efficacy measures (including Hb levels) and genotype-response correlations will be provided.
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 the 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 the establishment of HSCT guidelines and support future strategies, such as gene therapy.

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 sent to national hematopoietic stem cell transplantation (HSCT) registries, national and international databanks and to physicians involved in HSCT on PKD patients. The latter were asked to complete a questionnaire on disease characteristics, pre-transplant condition, transplant regimen and post-transplant outcome. Two additional cases were reported from a recently published report (Kim. 2016. Bone Marrow Transplantation).

Table 1.

Results: From 1996 to 2016 a total of 16 PKD-patients were reported to have been treated by stem cell transplantation. Eight patients were treated in the EU and eight in Asian centres, respectively. No patient resulted to be transplanted in the US. Median age at transplantation was 6.5 years. (10 patients (62.5%) were <10 years; 6 (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 splenectomy six months post transplantation. Two patients were lost from secondary graft failure. One of these had recovered a 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 sur-

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.

S453 HEREDITARY XEROCYTOSIS: CLINICAL AND BIOLOGICAL PRESENTATION AT DIAGNOSIS IN A RETROSPECTIVE SERIES OF 103 PATIENTS

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Background:

Hematopoietic Innovative Therapies Division, Centro de Investigaciones Energeticas, Medioambientales y Tecnologicas (CIEMAT), Centro de Investigaciones Biomédicas en Red de Enfermedades Raras (CIBERER), Madrid, Spain

Methods: This is an international case series. Queries were sent to national hematopoietic stem cell transplantation (HSCT) registries, national and international databanks and to physicians involved in HSCT on PKD patients. The latter were asked to complete a questionnaire on disease characteristics, pre-transplant condition, transplant regimen and post-transplant outcome. Two additional cases were reported from a recently published report (Kim. 2016. Bone Marrow Transplantation).

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Results: From 1996 to 2016 a total of 16 PKD-patients were reported to have been treated by stem cell transplantation. Eight patients were treated in the EU and eight in Asian centres, respectively. No patient resulted to be transplanted in the US. Median age at transplantation was 6.5 years. (10 patients (62.5%) were <10 years; 6 (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 splenectomy six months post transplantation. Two patients were lost from secondary graft failure. One of these had recovered a 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 sur-

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.
Sickle Cell Center, Medical College of Georgia, Augusta University, Augusta, Aims: This 2017;376:429-439).

Background: The median hemoglobin level was in the normal range: 135±19 g/L (range 71-195) with a slight macrocytosis (median MCV: 109±8fL) and a marked reticulocytosis (median reticulocyte count: 202±141 G/L). Of note, 57 patients (55%) presented a totally compensated hemolysis with a hemoglobin level above 115g/L. MCHC was in the normal range (median 335±1,3g/dL) but was above 66 g/dL for 28 (27,1%) patients. Stomatocytes were noticed on the blood smear in 42 patients over 70 available, numbered as rare (19%), few (60%) or numerous (21%). Genetics could be performed in 45 subjects from 22 distinct families. At least one PIEZO1 mutation was identified in very affected subjects. No KCNN4 mutations were found in these typical ektacytometric forms of HX.

Summary/Conclusions: This work represents the largest HX series and highlights the important heterogeneity in the clinical features at diagnosis. One important finding is that most patients were not anemic and presented a compensated hemolysis. In a significant percentage of cases, diagnosis was made in the exploration of extra hematological features including perinatal edema or hemochromatosis occurring despite the absence of any red blood cells transfusion. Moreover, we confirmed the very high risk of thrombotic events after splenectomy, underlining the absolute necessity of formally eliminating HX in any unexplained chronic hemolysis each time splenectomy is considered.

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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

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Background: Chronic hemolysis is a hallmark of sickle cell disease (SCD). Hemolysis in SCD has been associated with elevated levels of heme in the circulation of both human patients and SCD mice. It was shown that TNF-α-primed neutrophils form NETs in SCD, as treatment of SCD mice with the heme-binding protein hemopexin (Hpx) to scavenge free heme led to reduced NET formation by TNF-α-primed neutrophils. Free heme was suggested to mediate the formation of neutrophil extracellular traps (NETs) as shown by staining of lung sections (Chen et al. Blood 2014). In addition, the administration of DNase I to degrade NETs led to improved survival. Furthermore, it was shown that plasma from SCD patients obtained during SCD crisis induced NET formation by TNF-α-primed neutrophils. Free heme was suggested to mediate the formation of NETs in SCD, as treatment of SCD mice with the heme-binding protein hemopexin (Hpx) to scavenge free heme led to reduced NET formation (Chen et al. Blood 2014).

Methods: SUSTAIN was a randomized, double-blind, placebo-controlled, Phase II study (NCT01895361). Patients aged 16–65 years with SCD (including HbSS, HbSC, HbSβ0-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 for up to 50 weeks. At the final assessment visit at week 52, descriptive statistics were used to summarize the frequency of patients who were SCPC event-free for the duration of the study, based on the intent-to-treat (ITT) population overall and by prior SCPC events, SCD genotype and HU use at baseline.

Table 1.

| Table. SCPC event-free patients by prior SCPC events, SCD genotype and HU use |
|------------------|------------------|------------------|
| **SCPC events in the year prior to study** | **Crizanlizumab 5.0 mg/kg** | **Crizanlizumab 2.5 mg/kg** | **Placebo** |
| Z-4 | N=67 | 10/41 (24.4) | 10/41 (24.4) | | |
| 5-10 | 7/25 (28.0) | 2/25 (8.0) | 1/24 (4.2) | | |
| **Genotype** | **HbSS** | **HbSC** | **HbSβ0-thalassemia** | **HbSβ+–thalassemia** |
| Z-4 | 15/47 (31.9) | 9/47 (19.1) | 8/47 (17.0) | | |
| Other | 9/20 (45.0) | 3/19 (15.8) | 3/18 (16.7) | | |
| **HU use** | **Yes** | **No** | | | |
| Z-4 | 14/42 (33.3) | 2/42 (4.7) | | | |
| 5-10 | 9/42 (21.2) | 7/42 (16.7) | | | |
| **All data are n/N (%)** | | | | | |

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%, HbSβ0–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 (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). Additionally, 33.3% of patients who were taking HU and treated with crizanlizumab 5.0 mg/kg were SCPC event-free during the study, compared with 17.5% in the placebo arm, possibly suggesting an additive effect.

Summary/Conclusions: Treatment with crizanlizumab 5.0 mg/kg appears to increase the likelihood of adult patients with SCD being SCPC event-free while on treatment, even in high-risk subpopulations. Crizanlizumab 5.0 mg/kg was also effective in those who had experienced at least two SCPC in the previous year despite taking HU, suggesting that this dose is effective as a disease-modifying agent that meets an unmet medical need.
mine whether ex vivo Hpx addition to human SCD sera would prevent NET formation.

Methods: Patient serum and plasma samples were obtained from 32 incidents of VOC in 24 adult SCD patients, with informed consent. Moreover, steady state samples were obtained at least 4 weeks after discharge from the hospital. Patients having had a blood transfusion in the 3 months prior to admission were excluded. NET formation by human neutrophils from healthy donors was studied using confocal fluorescence microscopy and staining for extracellular DNA with the cell nonpermeable dye Sytox Green. The presence of extracellular DNA that stains positive for citrullinated histone H3 confirmed the formation of NETs (Figure 1A).

Results: Indeed, we found that hemin (ferriprotoporphyrin IX) activated neutrophils to generate reactive oxygen species and release NETs, which was prevented by addition of plasma-derived Hpx. Moreover, exposure of neutrophils to sera from patients with SCD promoted NET formation, which was significantly enhanced during VOC. However, we observed that circulating free heme levels were elevated in SCD patient serum irrespective of disease state, and serum concentrations of Hpx were reduced in both VOC and steady state compared to healthy donor serum. Strikingly, addition of Hpx in supraphysiological concentrations failed to prevent the formation of NETs in all SCD sera tested. We and others (Chen et al. Blood 2014) have found that, in contrast to hemo, protoporphyrin IX does not trigger NET formation, revealing that the iron atom is required for the release of NETs. This observation led us to investigate whether free iron may directly induce NET formation. When neutrophils were exposed to Fe-NTA or serum from a thalassemia patient with iron overload, NETs were formed. Scavenging of free iron by addition of the iron-chelator deferoxamine or the specific iron-binding protein apotransferrin prevented NET release (Figure 1B). Moreover, we found that sequestration of free iron prevented NET formation induced by a subset (6 out of 11 tested), but not all, sera of patients with VOC (Figure 1C and D). In addition, sickled red blood cells (RBCs) are known to bind to neutrophils in vitro. Here, we found that neutrophils released NETs in response to sickled RBCs, even in the presence of Hpx. By contrast, blocking of complement C5 activation completely prevented the formation of NETs when neutrophils were exposed to sickled RBCs (Figure 1E).

Summary/Conclusions: In summary, we observed that sequestration of free iron with these iron binding compounds may be explored therapeutically to prevent or treat VOC development in SCD. Finally, complement activation in the presence of sickled RBCs activates neutrophils to release NETs, which may also contribute to VOC and SCD pathogenesis. Therefore, anti-CS IgG may represent an alternative therapeutic strategy to prevent VOC in SCD.

New drugs for rescue in relapsed/refractory multiple myeloma

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 to receive 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 0.58, 0.86). Patients with t(4;14) or t(14;16) had a greater benefit (35% vs 26% risk of progression/death (HR 0.65; 95% CI 0.46, 0.94). ORR was greater with ELd vs Ld (79% vs 66%) and the duration of response benefit was maintained over time (HR 0.77; 95% CI 0.62, 0.95). Early separation of the Kaplan–Meier survival curves, which remained consistently separated over time, supports a sustained OS benefit in favor of ELd vs Ld (Figure). Grade 3-4 adverse events in >5% of patients were generally comparable between ELd and Ld arms-vascular diseases (10% vs 8%; mostly venous-related), second primary malignancies (SPMs; 9% vs 6%) and cardiac disorders (5% vs 8%); the exception was a

Figure 1. OS Kaplan-Meyer Curve (all randomized patients).
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A PHASE IB STUDY OF ISATUXIMAB PLUS POMALIDOMIDE (POM) AND DEXAMETHASONE (DEX) IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED EFFICACY AND SAFETY RESULTS FROM CASTOR, A MULTICENTER, RANDOMIZED, OPEN-LABEL, PHASE 3 STUDY OF ISATUXIMAB PLUS POMALIDOMIDE AND DEXAMETHASONE VERSUS BORTezomib AND DEXAMETHASONE (Vd) IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM) - UPDATED ANALYSIS OF CASTOR


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Background: In a phase 3 head-to-head comparison of two proteasome inhibitors (Pis) in patients with relapsed or refractory multiple myeloma (RRMM), ENDEAVOR, progression-free survival (PFS) was shown to be significantly longer with carfilzomib and dexamethasone (Kd) than with bortezomib and dexamethasone (Vd) (median 18.7 months vs 11.4 months). ENDEAVOR, progression-free survival (PFS) was shown to be significantly longer with carfilzomib and dexamethasone (Kd) than with bortezomib and dexamethasone (Vd) (median 18.7 months vs 11.4 months). ENDEAVOR, progression-free survival (PFS) was shown to be significantly longer with carfilzomib and dexamethasone (Kd) than with bortezomib and dexamethasone (Vd) (median 18.7 months vs 11.4 months). ENDEAVOR, progression-free survival (PFS) was shown to be significantly longer with carfilzomib and dexamethasone (Kd) than with bortezomib and dexamethasone (Vd) (median 18.7 months vs 11.4 months).

Methods: Patients who had RRMM and who had received 1–3 prior lines of therapy were randomized in a 1:1 ratio to receive Kd or Vd. In the Kd arm, carfilzomib was given on days 1, 2, 8, 9, 15, and 16 (20mg/m² on days 1, 2, 8; 14mg/m² on days 9, 15, 16, 22, and 23 of 28-day cycles. The Vd arm, bortezomib (1.3mg/m²) was given intravenously or subcutaneously on days 1, 4, 8, and 11 and dexamethasone 20mg was given on days 1, 2, 4, 5, 8, 9, 11, and 12 of 28-day cycles. Patients were treated until progression or withdrawal of consent. OS was compared between treatment arms using a stratified log-rank test.

Results: The median treatment duration was 48 weeks for carfilzomib (N=464) and 27 weeks for bortezomib (N=445), with a median follow up of 38 months for Kd and 37 months for Vd. The median OS (95% CI) was 47.6 (42.5-NE) months in the Kd arm and 40.0 (32.6-42.3) months in the Vd arm, and all-cause mortality was significantly reduced with Kd vs Vd (HR, 0.791; 95% CI, 0.648-0.964; 1-sided p=0.0100). The overall survival benefit was consistent regardless of prior bortezomib therapy (HR 0.75 for Kd vs Vd, no prior bortezomib; HR 0.84 for Kd vs Vd, prior bortezomib) and across all age groups (HR, 0.85 <65 yr; 0.71, 65-74 yr; 0.84, 75+y); baseline ECOG performance status (HR, 0.61 [1], 0.68 [2], 0.81 [3], 0.91 [4]; cytogenetic risk groups (HR, 0.83, high risk; 0.85, standard risk), and number of prior lines of therapy (HR, 0.83, 1 prior line; 0.76, 2-3 prior lines). The most frequent any-grade adverse events in the Kd arm were (Kd vs Vd) anemia (42.5% vs 28.3%), diarrhea (36.3% vs 40.6%), pyrexia (32.4% vs 15.4%), dyspnea (32.2% vs 13.6%), fatigue (32.2% vs 30.7%), and hypertension (32.2% vs 9.9%). Grade 3 or higher adverse events were experienced by 81.4% of patients in the Kd arm and 71.1% of patients in the Vd arm.

Summary/Conclusions: ENDEAVOR was the first randomized phase 3 trial to directly compare two different Pis in RRMM. Patients who received Kd had significantly longer OS compared with patients who received Vd. Safety results were comparable with those previously reported in the PFS interim analysis for ENDEAVOR.
Results: A total of 498 patients were randomized with median (range) age of 64 (30-88) years. Patients received a median (range) of 2 (1-10) prior lines of therapy; 66% of patients previously received bortezomib, and 21% were refractory to lenalidomide in their last prior line of therapy. After median follow-up of 13.0 months, DVD significantly prolonged PFS compared with Vd alone (median: 7.1 months, DVD: 18.3% vs 3.6%). Twelve-month PFS rates were 60% versus 22%, respectively. Significant PFS benefit was observed with DVD over Vd regardless of the number of prior lines of therapy, although the greatest benefit was seen in patients with 1 prior line of therapy (median: not reached vs 7.9 months; HR, 0.22; 95% CI, 0.14-0.34; P<0.0001). Overall response rate (ORR; 84% vs 63%) and rates of very good partial response (VGPR) or better (62% vs 29%) and CR or better (26% vs 10%) continued to be significantly higher with DVD compared with Vd (P<0.0001 for all). MRD-negative rates were more than 4 times higher at all 3 sensitivity thresholds with DVD versus Vd: 18.3% versus 3.6% at 10−4 (P<0.0001), 10.4% versus 2.4% at 10−5 (P<0.01), and 4.4% versus 0.8% at 10−6 (P<0.01). MRD-negative patients had prolonged PFS compared with MRD-positive patients at 10−4 sensitivity threshold (Figure). At the clinical cut-off date, 37 (15%) deaths in the DVD group and 58 (24%) in the Vd group have been observed (HR, 0.63; 95% CI, 0.42-0.96), and follow up is ongoing. Thrombocytopenia was the most common grade 3 or 4 treatment-emergent adverse event (45% with DVD vs 33% with Vd). No new safety signals were reported after median treatment duration of 11 months with daratumumab. Updated efficacy and safety data with longer follow up will be presented at the meeting.

Figure 1.

Summary/Conclusions: DVD is superior to Vd in terms of PFS, ORR, depth of response, and MRD-negative rates, with no new safety signals reported. These updated data further support the use of DVD as a standard care in RRM.

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 3 months) were longer for pts not refractory to any of these therapies versus refractory pts. ORR for pts with or without cytogenetic abnormalities, respectively, was as follows: 78% vs 65% for t(11;14), 60% vs 67% for t(4;14), 47% vs 73% for del(17p), and 63% vs 69% for del(13q).

Summary/Conclusions: VEN combined with bortezomib and dexamethasone has an acceptable safety profile with promising anti-myeloma activity, and the highest response rates were observed in R/R MM pts who were not refractory to PI or IMiDs. These data support the ongoing phase 3 trial with this regimen in R/R MM.
Improving prognosis 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: Reappraisal of definitions for CK in CLL and systematic investigation of clinicobiological associations and prognostic impact.

Methods: 3580 CLL and monoclonal B-cell lymphocytosis (MBL) patients (CLL=3322, 93% and MBL=258, 7%, respectively) were analysed with Cpg-G-oligodeoxynucleotides/interleukin 2 (CpG/IL2, n=379, 11%), phorbol-12-myristateacetate (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, TTP53: 829/2051 (40%)/del(13q), 1769/3271 (54%)/ trisomy 12, 507/3260, (79% and 57%, respectively). The median OS was 5.1 years for the high-CK cases versus (median: 8.75 years for the low-CK' cases (p<0.0001). We also identified 46 cases (12% of those with ≥3 aberrations) who carried +12,+19 plus other numerical and/or structural abnormalities and displayed extremely indolent clinical course (median OS not yet reached and only 4 deaths at a median follow-up of 5.2 years). When high-CK was assessed as an independent parameter, it was correlated with inferior OS in the univariate analysis, retaining significance also in the multivariate analysis (p=0.012) independently of the remaining parameters, including clinical stage, U-CLL and TP53 status. In contrast, low/intermediate-CK had no impact on OS, 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 axiomatically considered unfavorable in CLL, representing a heterogeneous group with variable clinical behavior. High-CK with ≥5 chromosomal aberrations emerges as prognostically adverse, independently of clinical stage, IG somatic hypermutation and TP53 status. Prospective clinical validation is warranted before finally incorporating high-CK in risk stratification in CLL.

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IS THE TREATMENT OF CHOICE FOR IGHV MUTATED CLL WITHOUT POOR FISH CYTOGENETICS?

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Background: Chemoimmunotherapy (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/or poor FISH cytogenetics (i.e. del(17p)) show a better outcome than those with unmutated IGHV genes (U-CLL), and/or poor FISH cytogenetics and display 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, par-ticularly FCR.

Aims: The aim of this study was to analyse the outcome of M-CLL patients with unfavorable cytogenetics according to the type of therapy.

Methods: We analysed 816 CLL patients from Sant Pau Hospital, Barcelona, Spain; Uppsala University Hospital, Sweden and IRCCS San Raffaele Scientific University, Milan, Italy for whom IGHV mutational status was available. Endpoints were OS and TFS.

Table 1.

Results: 488 patients had mutated IGHV genes (400 without unfavorable FISH cytogenetics; 28 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 64% (CI, 58-70) for U-CLL cases (p<0.05). TFS at 5 and 10 years was 73% (CI, 70-76) and 53% (CI, 48-58) and 61% (CI, 56-66) and 28% (CI, 23-33) 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-prognostic FISH cytogenetics, 13 with either del(11q) and/or del(17p) and 12 cases in whom FISH information was not available] and 263 U-CLL required therapy. Front-line treatments consisted of purine analogues (PA)-based therapy (n=83), alkylating agents (n=212), anti-CD20 moAbs with PA or bendamustine (n=75), anti-CD20 moAbs with alkylating agents (n=21), BCR-signal inhibitors or BCL2 antiapoptotic agents (n=9), others (n=23), and unknown (n=1).

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IBRUTINIB, FLUDARABINE, CYCLOPHOSPHAMIDE, AND OBINUTUZUMAB (GA101) 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 >90%) after receiving first-line FC. Aims: To develop an FC-based chemoimmunotherapy regimen of finite duration that included ibritinib and obinutuzumab. The intent was to limit FC to 3 courses, potentially reducing short- and long-term toxicity, while maintaining efficacy through the addition of ibritinib and a more potent antibody (obinutuzumab).

Methods: We designed an investigator-initiated phase II trial with ibritinib, fludarabine, cyclophosphamide, and obinutuzumab (iFCG) for previously untreated 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, and no comorbidities. Patients were randomized to receive 2 cycles of iFCG followed by 1 cycle of maintenance therapy, with ibritinib continued for a total of 5 cycles. Patients were evaluated for response at the end of each cycle and for MRD negativity at the end of induction.

Results: A total of 23 patients were enrolled from 10 centers. The median age was 59 years (range, 25-71); there were 18 men. Three patients were ineligible, leaving 20 evaluable patients. 15 patients had received prior therapy. At the end of induction, 14/20 achieved CR/CRi with MRD-negative status. 14/18 achieved MRD-negative remission in the marrow as assessed by next generation sequencing (NGS). 14/18 achieved MRD-negative remission in the marrow and 16/18 achieved MRD-negative remission in peripheral blood. Overall, 7/18 achieved CR/CRi with MRD-negative status in the marrow and 16/18 achieved MRD-negative remission in the marrow as assessed by NGS.

Summary/Conclusions: IFCG achieves high rate of MRD-neg remission after 3 courses. Pt enrollment continues, and updated results will be presented at the EHA meeting.

BENDAMUSTINE (B), FOLLOWED BY OBINUTUZUMAB (G, GA101) AND VENETOLACX (A, ABT-199) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): CLL2-BAG PHASE-II TRIAL OF THE GERMAN CLL STUDY GROUP (GCLLSG)

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Background: Based on the theoretical "sequential triple-T" concept [Hallek M., Blood 2013; 122(23): 3723-34] of a tailored and targeted treatment aiming for total eradication of minimal residual disease (MRD), the GCLLSG designed the CLL2-BAG trial.

Aims: This prospective, open-label, multicenter phase-II trial investigates a sequential treatment with a B debulking, followed by G and A as induction and maintenance therapy in an all-comer population of physically fit and unfit, treatment-naive (TN) and relapsed/refractory (R/R) CLL pts.

Methods: Pts with an absolute lymphocyte count (ALC) ≥25.000/µl and/or lymph node (LN) ≥5cm were to receive 2 cycles of B as debulking (70mg/m2 d1&2 q28 days), then ibritinib 420mg qd from cycle 3. The remaining 18 pts then received G and A as induction and maintenance therapy, with ibritinib continued for a total of 5 cycles. The primary endpoint is the overall response rate (ORR) at the end of induction therapy; secondary endpoints include MRD evaluations, safety and survival parameters. This primary endpoint analysis is based on uncleaned data, the final analysis will be presented at the meeting.

Table 1.

<table>
<thead>
<tr>
<th>ORR</th>
<th>N=18</th>
<th>Marrow MRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR/CR</td>
<td>11 (61)</td>
<td>7/11 (64) neg</td>
</tr>
<tr>
<td>PR</td>
<td>7 (39)</td>
<td>5 (50)</td>
</tr>
<tr>
<td>R/S/M</td>
<td>99 (100% neg)</td>
<td>7/9 (78%) neg</td>
</tr>
</tbody>
</table>

Results: Between May 2015 and January 2016, 66 pts were enrolled. Two R/R pts died of a sepsis and 1 TN pt discontinued due to toxicity during the first induction cycle; these 3 pts with <2 induction cycles were excluded from the analysis as predefined by protocol. 34 pts were treatment-naive and 29 had R/R CLL (median number of prior therapies: 2, range: 1-8). Median age was 59 (28-77) years, the median CIRS score was 2 (0-14) and 16 pts (25%) had a history of heart failure, 12 pts (18%) had a history of diabetes mellitus, and 14 pts (21%) had a history of chronic obstructive pulmonary disease. 13 pts (20%) had a history of hypertension, 1 pt had a history of myocardial infarction, and 1 pt had a history of stroke.

Summary/Conclusions: BAG achieves high rate of MRD-neg remission after 3 courses. Pt enrollment continues, and updated results will be presented at the EHA meeting.
ed (table 1); with an ORR of 97%, at the end of induction, the primary endpoint was met. MRD negativity (<10−5) 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 was 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 (45%) were GCT3-4 and 1 had a fatal grade 5 outcome (sepsis in 4th induction cycle). Most SAEs occurred in the R/R cohort (61 SAEs, 74%) and during the induction phase (63 SAEs, 76%). Most common SAEs were infections (27 in 16 pts; including 13 CTC°3-5) and hematological disorders (18 in 10 pts; CTC°3-4), followed by infusion-related reactions (6 in 6 pts), laboratory TLS (5 in 5 pts. during debulking), 1 in induction cycle 1 with G 2 in cycle 3 and 1 in cycle 4 with G and A) and ischemic coronary artery disorders (5 in 4 pts). No clinical TLS occurred.

Summary/Conclusions: With an ORR of 97% and a MRD negativity of 89% at the end of induction, this front-line treatment for B debulking, followed by G and A was very efficacious in a heterogeneous study population and well tolerated except for 3 fatal septicamae in R/R pts.

Background: IDELA is an oral PI3Kδ inhibitor approved in the EU for use with rituximab (R) or ofatumumab in patients (pts) with previously treated CLL. IDELA is currently under investigation as a single agent for relapsed (R) or with either del(17p) or del(11q) and is unsuitable for other therapeutics. Prior single arm studies have suggested that front line use of IDELA may be associated with an increased frequency of transaminase elevations compared to relapsed pts.

Aims: To describe: 1) the safety of IDELA plus rituximab in previously untreated CLL pts with del(17p) in this terminated study, and 2) the relation of key AEs and age.

Methods: Treatment-naive pts with CLL and confirmed del(17p) were treated in a single arm study with R 375mg/m2 IV weekly x 8 and IDELA 150mg PO BID continuously until disease progression or intolerability. Informed consent was obtained prior to study entry. The study was fully enrolled when terminated early due to infection related safety concerns observed in a pooled analysis of ongoing Phase 3 IDELA trials in front line/early line therapy; the planned independent efficacy analysis was not performed, but investigator assessment is available.

Results: 102 pts (median age, 66; range, 37-86) were enrolled between Aug 2015 and Jan 2016; 101 pts received IDELA R for a median (med) duration of 6.4 months (range, 0.7-17.0). The study was terminated in Mar 2016, <8 wks after dosing of the last enrolled pt. 77 pts (75.5%) remained on study at the time of study closure. The reasons for discontinuation from study were death (4.9%), study drug (4.9%), investigator discretion (4.9%), investigator withdrawal of consent (2.9%), other anticancer therapy (2.0%), and lost to follow up (1.0%). The investigator assessed response rate was 79%. 101 pts (99%) had adverse events (AEs); Gr ≥3 occurred in 80.4%, the most frequent Gr ≥3 AEs were ALT increased (27.5%), neutropenia (20.6%), infections (18.6%), and diarrhea (14.7%). Laboratory Gr ≥3 ALT and/or AST elevations were seen in 41.2%, with med time of onset of 8.1 wks (range 4.1-24.7). The med age of pts both with and without Gr ≥3 ALT/AST was 66 years, and the incidence of Gr ≥3 ALT/AST was similar in younger (43.9%, <65yr) and older (39.3%, ≥65yr) pts. Gr ≥3 diarrhea/coilits occurred in 17.1% of pts <65yr and in 14.5% of pts ≥65yr. Gr ≥3 AEs occurred in 37.7% (70%), most frequently due to transamnase elevations (37.3%), and diarrhea/coilits (15.7%). Discontinuation due to AEs occurred in 27% of pts, most frequently due to ALT/AST elevation (9.8%). Severe serious adverse events were reported in 46 (45.1%), including pyrexia (10.8%), diarrhea (11.8%). AEs of special interest included Gr ≥3 CMV reactivation (10%) of which 3 were CMV PJP and 3 had PJP (presumably on prophylaxis), Gr ≥3 febrile neutropenia in 5 (4.9%) and any grade pneumonitis in 5 (4.9%). Of the 5 pts with CMV, all were CMV IgG+ at screening on prophylaxis, Gr ≥3 febrile neutropenia in 5 (4.9%) and any grade pneumonitis in 5 (4.9%). Of the 5 pts with CMV, all were CMV IgG+ at screening on prophylaxis and 2 also were IgM+. There were 6 on-study deaths, 3 associated with infective causes and 1 due to distant heart failure.

Summary/Conclusions: In IDELA plus rituximab treated front-line CLL, the pattern of AEs was similar to that seen in relapsed CLL studies at similar duration of therapy, however the frequency of Gr ≥3 ALT/AST was increased compared to the relapsed setting. There was no significant effect of age on the risk of either ALT/AST elevations or diarrhea/coilits. The occurrence of CMV and PJP infections is consistent with current IDELA labeling and speaks to the potential benefit of risk mitigation through PJP prophylaxis and CMV monitoring during treatment. NCT02044822.
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Background: CC-122 is a cereblon modulating agent that degrades Aiolos and Ikaros, resulting in potent anti-lymphoma and immunomodulatory effects on T- and NK-cell function. Phase I clinical data revealed promising activity of CC-122 against follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Preclinical combination of CC-122 with obinutuzumab has shown synergy in FL and additive effects in DLBCL vs either single agent (Chiu. ASH 2015), supporting further study of this combination’s therapeutic potential.

Aims: The current phase Ib study (EUDRACT 2014-003333-26; NCT02417285) evaluated the safety and efficacy of CC-122 with obinutuzumab in 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 (MZhL) ≥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-c9, 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, 4 mg and CC-122 formulated capsules (F6) 3 and 4 mg were evaluated in separate cohorts. Primary endpoints included safety and tolerability, non-tolerated dose (NTD), and maximum tolerated dose (MTD). Response was assessed using the international Cheson 2007 criteria every 2 cycles to c6, every 3 cycles to c12, and every 6 cycles thereafter.

Results: As of January 12, 2017, 34 R/R B-cell NHL patients with DLBCL (n=19), FL (n=15), or MZhL (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/MZhL 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 toxicity (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 stage III/IV in 76% of patients. Of the 18 DLBCL patients, 8 had transformed FL. Of the 16 FL/MZhL 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 toxicity (DLT) of grade 4 neutropenia (CC-122 dose level of AIC 3mg); no dose was yet an NTD. Median CC-122 duration was 22 wks (range, 3-71) equivalent to 6 cycles (range, 1-18). CC-122 dose reduction or temporary interruption occurred in 10 (29%) or 26 (76%) of patients, respectively, primarily due to adverse events (AEs). Most patients (96%) had >1 wk of interruption due to AEs. The most common ≥10% grade 3/4 treatment-emergent AEs (TEAEs) were neutropenia (50%) and thrombocytopenia (21%). Fifteen patients (44%) had ≥1 serious TEAE, including 2 each of febrile neutropenia (related to CC-122), cytokine release syndrome (related to obinutuzumab), and pneumonia. Three deaths occurred during the study (2 PD; 1 AE). Overall response rate (ORR) was 59%, including 26% CR and 32% PR (Table 1). Median time to best response was 57 d, and median duration of response was not yet reached. In evaluable patients, 6-mo progression-free survival (PFS) was 63%.

Table 1.

Summary/Conclusions: The combination of CC-122 and obinutuzumab was well tolerated and demonstrates promising response rates and durable remissions in R/R patients with B-cell NHL. CC-122 doses of ≥3 mg and obinutuzumab (1000 mg) every 28 days (FL) or 21 days (DLBCL) for 6 cycles.

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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 II 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. A Phase Ib study to escalate pola to any substituting obinutuzumab (G) for R could improve outcomes. We report updated results from the Phase 1b/2 (P1b/2) study evaluating pola + BR or BG in R/R FL and DLBCL and the expansion cohorts evaluating pola + BG in R/R FL and DLBCL.

Methods: All pts provided informed consent to participate in the study and were treated with pola (1.5mg/kg) + B (90mg/m2) and R (375mg/m2) or G (1000mg) every 28 days (FL) or 21 days (DLBCL) for 6 cycles. Responses were assessed by modified Lugano criteria after 3 cycles, end of treatment (tx), and every 6 months for 2 years during follow-up (fu).

Results: As of 14 Nov 2016, 65 pts were enrolled: 24 pts (12 FL, 12 DLBCL) in P1b and 41 pts (20 FL and 21 DLBCL) in P2. In safety evaluable pts, FL pts (N=32) were median age 63 yr (37-86), 82% ECOG 0-1 and 6% ECOG 2, 44% FLIPI1 3-5, 78% Stage III/IV, 2 (1-7) median lines of prior tx, 38% refractory to last tx, 3% prior transplant. Among 44% FL pts who received ≥1 dose, the adverse events (AEs) that occurred in ≥20% of pts were: fatigue (67%), nausea (54%), diarrhea (45%), vomiting (42%), pyrexia (39%) and constipation (39%). As expected, grade (Gr) 3/4 cytophenias were common: neutropenia (34% FL, 28% DLBCL), thrombocytopenia (16% FL, 13% DLBCL). AEs leading to study interruption in 1 pt in 1 pt. In FL, 5% (24/32) had Gr 3/4 AEs and 41% (13/32) had serious AEs (SAEs). The only SAE occurring in ≥10% was infection (22%). The most common Gr 3/4 non-heme AEs were infection (16%) and neutropenia (9%); AEs led to study interruption in 8 pts. In DLBCL, 88% (28/32) had Gr 3/4 AEs and 63% (20/32) had SAEs. Most common Gr 3/4 non-heme AEs were febrile neutropenia (13%), fatigue (10%), and diarrhea (13%). SAEs occurring in ≥10% of pts were infection (33%) and pyrexia (22%). AEs led to study interruption in 19 pts and discontinuation in 8 pts. There were 13 deaths: 9 PD, 4 AE (all unrelated to tx). Responses by modified Lugano 2014 criteria are shown in Table1. Median duration of response (DoR) for FL P1b pts was 16 months (mo) (median fu 14.5 mo). Median DoR for FL P2 (median fu 6.5 mo) and DLBCL P1b/2 (median fu 13.7 mo P1b, 6.4 mo P2) have not been reached.

Table 1.

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.

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SINGLE AGENT ORAL SELINEXOR EXHIBITS DURABLE RESPONSES IN RELAPSED/REFRACTORY DSqćiukCG B-CELL Lymphoma (DLBCL) OF BOTH GCB AND NON-GCB SUBTYPES: THE PHASE 2B SADAL STUDY

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Background: Patients (pts) with persistent DLBCL after two or more lines of therapy have limited effective treatment options. The nucleolar export protein exportin 1 (XPO1) is upregulated in hematologic malignancies, including DLBCL, and has pleiotropic effects on tumorogenesis including functional downregulation of tumor suppressor proteins (TSPs) and increased export and translation of mRNAs for oncoproteins c-Myc and key survival proteins such as Bcl-2. Selinexor (SEL), an oral XPO1 inhibitor, causes sequestration of TSPs including p53, p21, and IκBα, the latter of which serves to suppress NF-κB transcription, along with reductions in c-Myc and Bcl-2 family proteins. In a Phase I clinical study, pts with relapsed/refractory (R/R) DLBCL treated with SEL monotherapy showed activity in pts with R/R DLBCL after ≥2 prior regimens. Methods: Pts with R/R DLBCL were randomized to 60 or 100mg of SEL twice weekly (8 doses) per 28-day cycle. Pts were stratified by DLBCL subtype (GCB or non-GCB). The primary objectives are to determine the ORR and evaluate the safety of 60 vs 100mg doses. Disease response was assessed by an Independent Central Radiological Review (ICRR), using the Lugano Classification (Cheson, 2014). Results: 72 pts were enrolled: 37 pts on 60mg (24 M/ 13 F, median age 71 yrs) and 35 pts on 100mg (23 M/ 12 F, median age 68 yrs). Both groups had a median of 3 prior treatment regimens. The most common related adverse effects (AEs) were fatigue (47%), nausea (46%), anorexia (42%), and vomiting (33%). Common Grade 3/4 AEs were: thrombocytopenia (39%), fatigue (18%), neutropenia (18%), and anemia (13%). These were managed with dose interruption/reduction, platelet stimulators, and/or standard supportive care. Grade 3/4 fatigue (26% v 11%) and thromboctopenia (48% v 32%) were higher in the 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. Summary/Conclusions: SEL monotherapy shows activity in pts with R/R DLBCL including in pts with GCB subtype. 60mg SEL twice weekly was more tolerable than 100mg twice weekly, with fewer interruptions due to toxicity. Objective responses to SEL were durable at 60mg BIW, suggesting these responses were associated with clinical benefit.

Table 1. Independent Central Radiological Review-Best Response.

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>ORR (%)</th>
</tr>
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<tbody>
<tr>
<td>All</td>
<td>65</td>
<td>31 (47.7%)</td>
<td>7 (10.8%)</td>
<td>27 (41.5%)</td>
<td>8 (12.3%)</td>
<td>39 (59.2%)</td>
</tr>
<tr>
<td>60mg</td>
<td>32</td>
<td>20 (62.5%)</td>
<td>5 (15.6%)</td>
<td>6 (18.7%)</td>
<td>3 (9.3%)</td>
<td>28 (87.5%)</td>
</tr>
<tr>
<td>100mg</td>
<td>33</td>
<td>11 (33.3%)</td>
<td>2 (6%)</td>
<td>15 (45.5%)</td>
<td>6 (18.2%)</td>
<td>16 (48.5%)</td>
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<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>SD (%)</th>
<th>ORR (%)</th>
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<tr>
<td>Non-GCB Subtype</td>
<td>35</td>
<td>18 (51.4%)</td>
<td>6 (17.1%)</td>
<td>9 (25.7%)</td>
<td>6 (17.1%)</td>
<td>7 (20%)</td>
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</table>

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L-MIND: MOR208 COMBINED WITH LENALIDOMIDE (LEN) IN PATIENTS WITH RELAPSED OR REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA (R-R DLBCL)—A SINGLE-ARM PHASE II STUDY


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Background: The Fc-enhanced CD19 antibody MOR208 and the immunomodulatory drug LEN have demonstrated single agent activity in patients with R-R DLBCL. MOR208 and LEN have shown synergy in vitro and in vivo in preclinical lymphoma models.

Aims: This ongoing phase II study was designed to assess the safety and efficacy of MOR208 plus LEN in patients with R-R DLBCL.

Methods: Patients >18 years of age with R-R DLBCL, ECOG performance status 0–2, adequate organ function, having previously received at least 1 but not more than 3 prior therapies, including at least 1 CD20-targeting regimen and who are not candidates for autologous stem cell transplant (ASCT), are eligible. Treatment comprises up to 12, 28-day cycles of MOR208 12mg/kg IV, administered weekly during cycles 1-3 (loading dose day 4 of cycle 1) and every second week during cycles 4-12 plus LEN 25mg administered po days 1-21 of each cycle. Patients progression-free after 12 cycles receive up to 12 additional cycles of MOR208 12mg/kg IV, administered every second week. The primary endpoint is the overall response rate (ORR) by central radiology assessment. Secondary endpoints include disease control, duration of response, progression-free and overall survival, safety, and response by cell of origin and other biomarkers. A preplanned safety evaluation was undertaken.

Results: 31 of 80 planned patients were enrolled prior to data cutoff (3 January 2017). Median age was 74 years (range 47–82); 45% of patients received ≥2 prior lines of therapy; 23% had rituximab refractory disease; 74% had Ann Arbor stage III disease; 65% had elevated lactate dehydrogenase level, and 52% had a poor revised International Prognostic Index (3–5). The most common treatment-emergent adverse events (any grade/grade ≥3 [% patients]) were neutropenia (39/26), anemia (23/0) thrombocytopenia (16/6), infections (26/10) diarrhea (13/0), pyrexia (13/0), and rashes (13/6). Of 26 response evaluable patients (median follow-up 3.3 months), ORR (investigator assessed) was 58% (15 patients), with 7 (27%) complete responses. Median time to response was 1.8 months.

Summary/Conclusions: The combination of MOR208 plus LEN is well tolerated and shows promising activity in patients with R-R DLBCL. Accrual and follow-up of patients is ongoing, as are cell of origin and other biomarker analyses.
ENASIDENIB (AG-221) IN MUTANT-IDH2 RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA (R/R AML): RESULTS OF A PHASE 1 DOSE-ESCALATION AND EXPANSION STUDY

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Background: Recurrent mutations in isocitrate dehydrogenase 2 (mIDH2) occur in ~12% of AML patients (pts), mIDH2 proteins synthesize an oncometabolite, 2-hydroxyglutarate (2HG), causing DNA and histone hypermethylation and blocked myeloid differentiation. Enasidenib (AG-221) is an oral, selective, small-molecule inhibitor of mIDH2 proteins. Differentiation of myeloblasts, not cytotoxicity, appears to drive the clinical efficacy of enasidenib. In preclinical studies, bone marrow blasts from pts with mIDH2 exposed to enasidenib ex vivo were shown to produce mature, fully functioning neutrophils with conserved mIDH2 allele frequency, indicating differentiation of mature cells from the mIDH2 blasts (Yen et al, Cancer Discov, 2017). Additionally, no apoptosis was observed in mIDH2-R1402h erythroleukemia (TF-1) cells treated with enasidenib for 7 days in vitro.

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

Methods: This phase 1/2 study included pts aged ≥18 years (yrs) with mIDH2 WHO-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 investigational 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 Tx. Overall response rate (ORR; complete remission [CR] + CR with incomplete remission — myeloid- and megakaryocytic-fusion — or complete remission with incomplete blood count recovery 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 29 (82%) by cycle 7. Median duration of CR was 8.8 mos. ORR with enasidenib 100mg/day was 38.5% (Table). Seventeen pts (11%) proceeded to stem cell transplant. Response was associated with cellular differentiation, typically with no evidence of aplasia. Median overall survival (OS) of R/R AML pts was 9.3 mos. For pts who attained CR, OS was 19.7 mos. Pts who had received ≥2 prior AML Tx had a median OS of 8.0 mos.

Summary/Conclusions: Enasidenib was well tolerated, induced CRs in R/R AML pts, and was associated with OS of >9 mos in pts who had failed prior AML Tx. A randomized phase 3 study of enasidenib vs conventional care in older pts with late-stage R/R AML is ongoing (NCT02577406).

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SAFETY AND EFFICACY OF VENETOCLAX (VEN) IN COMBINATION WITH DECITABINE OR AZACITIDINE IN TREATMENT-NAIVE, ELDERLY PATIENTS (≥65 YEARS) WITH ACUTE MYELOID LEUKEMIA (AML)

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Background: Newly diagnosed patients (pts) with AML aged ≥65 years and ineligible for standard induction therapy have limited treatment options, and low overall survival. VEN is an orally bioavailable, selective BCL-2 inhibitor that has displayed single-agent activity in pts with relapsed/refractory AML. VEN at escalating doses combined with hypomethylating agents (HMAs) has demonstrated antileukemic activity, with an overall response rate (ORR) including complete remission [CR], and CR with incomplete marrow recovery of 60%. Combining VEN with HMAs, such as decitabine (DEC) or azacitidine (AZA), may provide a novel low-intensity approach for treating AML. Preliminary results from the expansion stage of a phase 1b trial comparing 2 doses of VEN plus either DEC or AZA (NCT02203773) are reported.

Aims: To evaluate the safety and efficacy of VEN at 400-mg vs 800-mg doses plus DEC or AZA.

Methods: This open-label, nonrandomized, two-stage phase 1b study evaluated the safety and efficacy of VEN plus DEC or AZA in treatment-naive pts ≥65 years of age with AML. Eligibility 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 VEN at 175 mg/m2/d (Arm F); 175 mg/m2/d, 250 mg/m2/d, and 312.5 mg/m2/d (Arm G) respectively. Grade 3-4 adverse events and clinical laboratory tests were recorded through cycle 2. The MTD was defined. The dose expansion stage consisted of 2 VEN dose cohorts (continuous 400-mg and interrupted 800-mg dosing) in each arm (D1, D2, E1, and E2, respectively) to determine optimal dose. Tumor lysis syndrome (TLS) prophylaxis was administered in C1 to all pts during VEN dose ramp-up until final dose was reached. All pts provided informed consent.

Targeted treatment of AML

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Results: As of 13/09/16, 100 pts were enrolled in the expansion stage; 25 pts in each arm. Overall, 61% pts were male; 50% had ECOG PS 0; 15% ECOG PS 1; and 35% ECOG PS 2; median age was 73.9 (range 65–86); 53% had adverse karyotype; and 22% had secondary AML. Median time on study was 6 (4-9), 6 (0.2–9), 5 (0.5–9), and 4 (1–8) mo for arms D1, D2, E1, and E2, respectively. The incidence of adverse events (AEs) was generally comparable between the 4 arms. Overall, the most common treatment-emergent AEs (TEAEs; in ≥30% of pts) were nausea (59%), diarrhea (42%), febrile neutropenia (FN; 41%), constipation (39%), fatigue, and decreased white blood cell count (31% each). The most frequent grade 3/4 TEAE and serious AE was FN (41% and 29%, respectively). No TLS was observed. Overall, 29 pts discontinued the study for ≥1 reason, including progressive disease (PD) per protocol (n=10), “other” (n=10; 9/10 proceeded to stem cell transplantation) and AEs not related to progression (n=10). A total of 16 deaths occurred; 12 pts died within 30 d of initiating VEN and HMA due to AEs (n=12) and PD (n=1). The ORR was 68%, with rates of 76% (19/25), 71% (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.

Figure 1.

Summary/Conclusions: Overall, the safety profile was favorable when combining VEN at either dose with DEC or AZA in treatment-naïve elderly AML pts. Promising activity with high ORRs was observed at the lower 400-mg VEN dose in both HMA arms. A Phase 3 study of VEN plus AZA is planned.

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% pts) including cytopenias) were nausea (72%), hypokalemia (46%), diarrhea (44%), fatigue (43%), and decreased appetite (41%). Grade 3/4 AEs (≥10% pts) were febrile neutropenia (34%), hypokalemia (15%), hypophosphatemia (13%), and hypertension (10%). No pts had clinical TLS; 1 pt had laboratory TLS, which was managed. The 30-d and 60-d mortality rates were 3% and 15%, respectively. The CR/CRi rate was 54% (33/61; 21% CR and 33% CRi). The overall response rate (ORR; CR+CRi+partial remission) was 61% (37/61). VEN+LDAC was shown to be active across a wide range of cytogenetic mutations and pt profiles (ORR: 70% in pts ≥75 years; 52% in 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.
reduced 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), 5 (8%) had stable disease >6 months, and 24 (38%) had progression. 3 pts are too early for response assessment (<3 courses). The median number of courses to CR/CRi/HI was 2 (range, 1-4+). The median OS among the CR/CRi/HI patients was 15.3 months (range, 2.29-16.64), HI pts was 5.0 months (range, 4.67-17.45+), and NR was 5.0 months (range, 0.29-16.16). The 4- and 8-week mortality were 5% and 11%, respectively. The median OS for the 63 evaluable pts on AZA+nivolumab compares favorably to historical median OS with AZA-based salvage protocols in similar pts treated at MDACC (P=0.10) (Fig 1A and Fig 1B). Grade 3/4 and Grade 2 immune toxicities were observed in 8 (12%) and 7 (11%) pts, respectively. The most common Grade 3/4 AEs on treatment included pneumonitis, colitis, nephritis, skin rash, and hypophysitis. One pt died from grade 4 pneumonitis/epiglottitis. In the remaining 14 cases the toxicities responded rapidly to steroids and 13 of these pts were successfully rechallenged with nivolumab. Time to onset of toxicities ranged from 4 days to 3.5 months. Multicolor flow-cytometry studies and Mass-cytometry (CyTOF) studies are conducted by the Immunotherapy Platform on baseline and on-treatment BM aspirate (end of cycle 1, 2, 4, 8). Baseline and end of cycle (EOC) 1 and 2 BM was evaluated in 6 responders and 19 non-responders. Pts who achieved a response had a baseline higher live total CD3+ (P=0.10), CD8+ T-cells (P=0.02), and lower live CD4+ Foxp3+PD1+ T-regulatory (T-reg) cells (P=0.01) infiltrate in BM. Patients who had a response had progressive increase in BM CD3+ cells and BM CD8+ cells, with increased ICOS (activation) marker on BM CD4-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.

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

**Summary/Conclusions:** Full dose AZA and nivolumab are tolerable and produce an encouraging response rate with durable responses in relapsed AML with poor risk features. Immune mediated toxicities occur and may be adequately managed with early recognition and systemic steroids. Up-regulation of CTLA4 may be a mechanism of resistance to PD1 based therapies in AML and suggest role for combination therapy.

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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, selectively FLT3-ITD inhibitor that confers a median overall survival (mOS) of 23 weeks and remission rate of 46% in a single-arm phase 2 study (AC220-002) in pts with AML with a FLT3-ITD mutation who were relapsed or refractory (R/R) to second line therapy. (Levis, et al, ASH 2012) As context, a study of AML pts, regardless of FLT3 mutation status, receiving second-salvage therapies showed 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.

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**Figure 1.** OS with AZA+Nivo compared to historical survival with AZA-based salvage protocols in similar pts treated at MDACC in (a) all salvage and (b) first relapse only
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 LYMPHOBластIC LEUKEMIA (ALL): UPDATE TO THE INTERIM ANALYSIS


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Background: The CD19-targeted chimeric antigen receptor (CAR) T-cell therapy CTL019, an investigational therapy that reprograms cytotoxic T cells to eliminate target cells, resulted in high response rates and a manageable safety profile in pediatric/young adult patients (pts) with R/R B-cell ALL in a single-center trial. Aims: We report an updated interim analysis from the first multicenter global pivotal pivotal phase 2/3 trial (ELIANA) of CAR T-cell therapy (ELIANA; NCT02435849) including data for 68 pts infused with CTL019, 50 of whom were followed for ≥6 mo. Methods: This is a single-arm, open-label, multicenter, global, phase 2 study of CTL019 in pediatric/young adult pts with CD19+/R R/B-cell ALL with ≤5% bone marrow lymphoblasts by morphology. CTL019 was manufactured from leukapheresed autologous peripheral blood T cells at a centralized manufacturing facility. The primary endpoint was overall remission rate (complete remission [CR] or 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, 0.30 μg/kg; range, 0.25-6.4 μg/kg) which was infused over 28d. Pts were evaluated for a protocol-specified algorithm including day 6-6 mo of infusion. Median age was 12 y (range, 3-23 y); 59% of pts had prior allogeneic stem cell transplant (alloSCT). Five infused patients had not reached 3 mo of follow-up; among 63 evaluable pts, 52 (83% [95% CI, 71%-91%]) achieved CR/CRi within 3 mo of CTL019 infusion, all of whom had minimal residual disease–negative marrow. The relapse-free probability at 3 mo of follow-up was 75% (95% CI, 57%-87%; median DOR not reached). The probability of survival was 99% (95% CI, 77%-94%) at 6 mo and 79% (95% CI, 63%-89%) at 12 mo. Seven pts (13% of responders) proceeded to alloSCT within 6 months while in remission. Cytokine release syndrome (CRS) was graded using the UPenn scale and managed using a protocol-specified algorithm; CRS occurred in 78% of pts (21% grade 3; 27% grade 4); no CRS-associated deaths occurred. 38% of pts received tocilizumab for treatment of CRS or without other anti-cytokine therapy. Most common grade 3/4 nonhematologic AEs (>5%) other than CRS were hypotension (22%), hypoxia (18%), and inflammatory cytokine syndrome (18.4%). The most common grade 3/4 neurologic AEs, with no grade 4 events and no cerebral edema reported. Grade 3/4 neuropenia with high (>38.3°C) fever occurred in 60% of pts. 2 pts died within 30 days of infusion (ALL progression, n=1; cerebral hemorrhage, n=1), and 9 pts died >30 days after infusion (ALL relapse/progression, n=6; HHV-6 encephalitis, pneumonia, systemic mycosis, n=1 each). CTL019 expansion in vivo correlated with CRS severity, and persistence of CTL019 along with B-cell aplasia in peripheral blood was observed for ≥1 y in some responders. Summary/Conclusions: The ELIANA study confirmed the efficacy of a single infusion of CTL019, without additional therapy, observed in a previous interim analysis and a prior single-center CTL019 trial. AEs were effectively and reproducibly managed globally by appropriately trained personnel at study sites. S477

CTL019 CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS IN PEDIATRIC PATIENTS (PTS) WITH RELAPSED OR REFRACTARY (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 CD19expressing 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, AUC28-day (exposure)-response analysis and impact of intrinsic/extrinsic and manufacturing factors on CTL019 expansion. Methods: Cellular kinetic parameters of CTL019 post infusion were derived using traditional pharmacokinetic principles and reported by response category (complete response [CR]/CR with incomplete blood count recovery [CRi] vs no response [NR]) using 2 assays of peripheral blood cells: qPCR and flow cytometry. AUC28-day-response relationships were evaluated using logistic regression. Relationships between manufacturing specifications, therapies for cytokine release syndrome (CRS) management, and anti-CAR19 antibodies on cellular kinetics explored using summary statistics and graphical- and model-based analyses.

Figure 1. Results: Data from 79 pts (ELIANA, n=50; ENSIGN, n=29) were pooled for analysis. Using qPCR, pts with CR/Cri (n=62) had ≥2-fold higher CTL019 expansion than pts with NR (n=7) (Cmax, 73.5% higher geometric [geo] mean; AUC28, 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 AUC28 with the exception that pts with a higher tumor burden at enrollment generally had higher expansion, based on box plots. 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. AUC28 increased with pres...
ence and severity of CRS. Pts who received anti-cytokine agents for grade 3/4 CRS also had higher expansion. CR/CRI pts treated with tocilizumab and steroids (n=17) had 89% higher AUC0-28d 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 transgene levels and CAR surface expression in peripheral blood (r²=0.592) by qPCR and flow cytometry, respectively, when matched by time points from the cellular kinetic profile. Slower B-cell recovery was observed in pts with AUC0-28d above the median. Post-dose anti-CAR19 antibody responses were determined from the fold change of anti-CAR19 antibodies above the baseline pre-dose value. Pts with treatment-induced or boosted anti-CAR19 antibody responses generally had lower expansion, based on box plots, compared with pts with treatment-unaffected anti-CAR19 antibody responses, although AUC0-28d was variable. The boosted levels of anti-CAR19 did not impact clinical response or relapse.

Table 1.

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 abort CTL019 proliferation. Cellular kinetics are important to understand the determinants of tumor response with CAR T-cell therapy.

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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 (CTX). For patients who fail 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 cells and CD19+ B cells to induce tumor cell lysis. In a randomized phase 2 trial of blinatumomab vs investigator’s choice of 4 standard 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/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.

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.

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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 or without full hematologic recovery (CR/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.
Background: ETV6-RUNX1 fusion is a common genetic aberration in childhood acute lymphoblastic leukemia (ALL) and is associated with good prognosis in the context of contemporary treatment regimens. The required treatment intensity for this well-described biologic subgroup with low risk of relapse is not known so far. In trial AIEOP-BFM ALL 2000, feasibility of reduced delay of intensified treatment to reduce the burden of chemotherapy was tested in a randomized approach in the standard-risk group. Treatment reduction was not successful in the total cohort (8-year probability of disease-free survival (8y-pDFS, ± standard error) 89.2±1.3% for reduced intensified treatment, 92.3±1.2% for the standard treatment (log-rank \(P=0.04\)) due to evidence of more relapses observed in patients treated less intensively. Aims: The retrospective subgroup analysis presented here focuses on the ETV6-RUNX1-positive patients included in the group of randomized standard-risk patients. Methods: From 07/2000 to 06/2006, 4741 eligible patients with ALL (age range 1-17 years) were enrolled in the trial AIEOP-BFM ALL 2000 (NCT 00403118 (BFM) and NCT 00813457 (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 \(\tau\)/cell receptor gene rearrangement polymerase chain reaction). They were randomly assigned to either receive the reduced-intensity protocol P-III or the standard protocol II (P-II) for delayed intensification. P-III is shorter than P-II (duration 29 vs 49 days), the dose of dexamethasone in P-III \(=0.74\). Cumulative incidence of relapse at 8 years was \(3.3±1.3\%\) and \(4.3±1.6\%\) (Gray = 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 not be as effective in the defined biologically defined subgroup. However, the present data is not result of a sufficiently powered non-inferiority study question focused on the subgroup of ETV6-RUNX1-positive patients, but reflects a subgroup analysis with descriptive character. Therefore, any decision for treatment reduction should be considered carefully.
mal translocation t(9;22) that gives rise to the oncogenic tyrosine kinase Bcr-Abl. Implementation of tyrosine kinase inhibitor (TKI) therapy resulted in significant clinical success but with TKIs failing to eradicate the disease initiating leukemic stem cell population (LSC), this treatment is not curative in the vast majority of patients. By using a transgenic CML mouse model, we previously showed that LSC persist despite complete Bcr-Abl kinase inhibition due to a lack of selective pressure. Subsequently, we identified the ITIM carrying Fc gamma receptor IIb (FcγRIIb; CD32) to be 2.8-fold upregulated in Bcr-Abl+ versus control LSK (lin−;Sca-1+;c-kit+) cells using microarray and qRT-PCR.

**Aims:** In this study, we first aimed to validate Bcr-Abl mediated FcγRIIb upregulation on mRNA and protein level in leukemic cells. Next, we tested the effect of shRNA-mediated FcγRIIb knock-down and depletion on CFCs (colony forming unit) capacity, proliferation and leukemic signaling in vitro. Finally, we studied the disease-initiating potential of primitive CML stem and progenitor cells upon FcγRIIb knock down.

**Methods:** qRT-PCR and western blot analyses were applied using cell lines, primary BM cells, and Foxn68 immortalized murine bone marrow (BM) cells for studying FcγRIIb expression and signaling. In order to test the biology of CML cells in vitro, we performed CFU and proliferation assays. Moreover, we performed viral infection of S-FU treated SCILTA/Bcr-Abl BM using FcγRIIb-shRNA or scrambled control and subsequent transplantation, followed by analyses of the disease, including immune-phenotyping, RNA and protein expression as well as histological analysis.

**Results:** Bcr-Abl increased FcγRIIb mRNA (13.2-fold, p<0.001) and protein expression in primary murine lineage negative (lin−) BM cells. Reduction of FcγRIIb in immortalized SCILTA/Bcr-Abl progenitor cells significantly reduced CFU and promoted differentiation (10.6-fold, p<0.001) upon FcγRIIb knock down. Moreover, transplantation of SCILTA/Bcr-Abl shRNA:FcγRIIb BM cells (CD45.1+) into FVB/N wildtype (WT) CD45.2+ recipients reduced spleen weight (352 ± 59.13mg), as compared to scrambled shRNA (536.1 ± 101.72mg). FACS analysis revealed a decrease in GFP+;CD45.1+ BM cells (1.43-fold, p<0.001) upon FcγRIIb knock down. Likewise, donor-derived Gr-1+ cells (Gr-1++;CD45.1++;GP+F) were reduced in the BM (1.28-fold, p<0.001) of these mice. Flow-cytometric analysis of the stem cell compartment revealed decreased leukemic BM LSK cells (lin−;c-kit−;Sca-1−;CD45.1−; GP-, 1.38-fold, p<0.05) in mice transplanted with shRNA:FcγRIIb vs scrambled control. We also observed similar effects upon FcγRIIb depletion (FcγRIIb−/−) vs wildtype (FcγRIIb+/+), combined with virally induced Bcr-Abl expression. Interestingly, Bcr-Abl signaling induces FcγRIIb phosphorylation in leukemic cells. Analysis of downstream signal pathways showed decreased levels of p-ERK, p-BTK, p-PLCγ1 in FcγRIIb−/−, compared to FcγRIIb+/+ Bcr-Abl transduced immortalized SCLtTA/Bcr-Abl BM cells.

**Summary/Conclusions:** FcγRIIb is upregulated in LSC derived from transgenic CML mice upon Bcr-Abl expression. Complete depletion or knock down of the receptor reduces CFU capacity and cell growth in CML cells and significantly impairs CML development and LSC burden in vivo, presumably due to impaired leukemic downstream signaling. Our data demonstrate that FcγRIIb is critical and disease specific making it a potential novel therapeutic target in CML stem cells.

**S482**

**MYC-DEPENDENT REPRESSION MECHANISM OF THE MiR-150 TRANSCRIPTIONAL REGULATION IN CHRONIC MYELOID LEUKEMIA**

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**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, the degree of amplification bias, the absolute quantification of 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 assay reproducibility of the Q-PCR methods we selected patients that 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 with digital PCR and control gene expression were analyzed by digital PCR (dPCR, n=19) 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. The mean difference was -4.85 ± 1.56 log 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 IS at baseline was 58% (range, 2.4% - 487%) versus 93% by DNA methods (range, 2.4% - 235%). Interestingly, BCR-ABL1 DNA was significantly higher than mRNA at 1, 2, and 3 months (Figure). There was good agreement between positive results from 6 months of TKI therapy onwards (mean bias -0.02: 95% limits of agreement from -1.15 to 1.11). Comparing the limit of detection, BCR-ABL1 DNA was detectable in 60/148 (41%) samples with undetectable mRNA.
Aims: The first phase of the study was aimed to i) create a network of 4 labs sharing a common protocol, a joint database for clinical and mutational data and a common pipeline of data analysis, interpretation and reporting, and ii) verify accuracy and inter-laboratory reproducibility of results. The second phase of the study, involving 39 Italian Hematology Units, was meant to prospectively assess the frequency of low burden mutations in CML pts with Failure or Warning to any TKI.

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; NIT, 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; NIT, n=1; 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 Sanger Seq and 52/159 (33%; 44 Failures and 8 Warnings) by Deep Seq. Among the pts with low burden mutations detectable by Deep Seq, 4 had a T315I; 34 had other known TKI-resistant mutations; 14 had only mutations with unknown clinical significance. Pts positive for mutations by Deep Seq were more frequent in the High and Intermediate Sokal risk group. The number of positive pts and the number of mutations per pt were not significantly higher in those receiving 2nd- or subsequent-line TKI therapy than in those receiving 1st-line TKI therapy. Compound mutations were 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 18%); 3) all the pts who need to be switched to another TKI would benefit from sensitive BCR-ABL1 KD mutation screening by Deep Seq.

Figure 1.

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

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ESTABLISHING A NATIONAL NETWORK OF LABORATORIES USING NEXT GENERATION AMPLICON DEEP SEQUENCING FOR ROUTINE 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, multinational 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 sharing a common protocol, a joint database for clinical and mutational data storage and a common pipeline of data analysis, interpretation and reporting, and ii) verify accuracy and inter-laboratory reproducibility of results. The second phase of the study, involving 39 Italian Hematology Units, was meant to...
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

Methods: Patients with persistent cytopenia who did not fulfill the criteria for MDS are now classified as idiopathic cytopenia of undetermined significance (ICUS). This study was designed to investigate if sequencing of the cohort could bring additional information in regards to overall survival.

Results: So far we included 157 patients, 122 were classified as ICUS and 35 as MDS and the median age is 65 and 68 years, respectively (p=0.27). We have sequenced 78% of the ICUS patients and 74% of the MDS patients. In total 53% and 73% of the ICUS and MDS patients had at least one mutation detected, respectively. If the patients carried a mutation, the median number of mutations was two in both the CCUS and the MDS group. The most commonly mutated genes were TET2, SRSF2, DNMT3A and ASXL1 in 38 patients (31%), n=16 (13%), n=10 (8%), n=10 (8%), respectively. There were no significant differences in the distribution between the two groups. Mutations in NRAS, KRAS, TP53 were only identified in one patient each. The overall survival between the ICUS and the low-risk MDS patients did not differ (p=0.18) (figure 1). We also subdivided the ICUS patients into non-clonal ICUS and CCUS, but observed no difference between these two groups (p=0.355).

Eight of the patients categorized as ICUS progressed to a myeloid neoplasm during the follow up, and of these seven had a detectable mutation at time of enrollment, only one ICUS patient without a detectable mutation progressed (p=0.06).

Summary/Conclusions: We here demonstrate that low-risk MDS and ICUS patients share similar survival patterns, however, larger studies with longer follow up are needed. Mutations are most commonly found in the epigenetic regulators in this cohort of ICUS and low-risk MDS, while mutations in classical tumor suppressors and oncogenes such as TP53 and NRAS are rare. Mutational screening seems promising in identifying patients at risk of progression, however, other biomarkers for prognostication are warranted.

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AN UPDATE OF A PHASE II STUDY OF NIVOLUMAB (NIVO) OR IPILIMUMAB (IPI) WITH AZACITIDINE IN PTS WITH PREVIOUSLY TREATED OR UNTREATED MYELODYSPLASTIC SYNDROMES (MDS)

Methods: We designed a phase II study of Nivo or Ipi in monotherapy or combination for pts with MDS. Pts with prior therapy with HMA were to be treated in one of 3 consecutive cohorts: 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 at least one mutation having retained or developed over time. The stopping rule for toxicity was not met in any of the cohorts. Delays of therapy due to AEs were required in 9 pts due to: rash (N=1), adrenal insufficiency (N=1), colitis (N=1), thyroiditis (N=2), pneumonitis (N=3), and nephritis (N=1). Early 8-week mortality occurred in 1 patient due to non-related intracranial hemorrhage. The ORR was 80% (13/21) in the AZA+Nivo cohort including 6 CR. The ORR was 0% and 30% (5/18) in the Nivo and Ipi arms, respectively.

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

Methods: 63 pts were randomized into 3 consecutive cohorts combining AZA 75mg/m² iv daily days 1-5 of a 28 day cycle with: cohort #1: Nivo 3mg/kg iv days 1 and 15 of a 28 day cycle; cohort #2: Nivo 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 at least one mutation having retained or developed over time. The stopping rule for toxicity was not met in any of the cohorts. Delays of therapy due to AEs were required in 9 pts due to: rash (N=1), adrenal insufficiency (N=1), colitis (N=1), thyroiditis (N=2), pneumonitis (N=3), and nephritis (N=1). Early 8-week mortality occurred in 1 patient due to non-related intracranial hemorrhage. The ORR was 80% (13/21) in the AZA+Nivo cohort including 6 CR. The ORR was 0% and 30% (5/18) in the Nivo and Ipi arms, respectively.

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.
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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Background: Hypermethylating agents (HMA) such as azacitidine and decitabine remain the standard of care for the treatment of myelodysplastic syndromes (MDS) however, loss of response to therapy is associated with poor outcomes. Multiple studies have tried to identify biomarkers of response but the impact of the mutational architecture present at the time of diagnosis in response outcomes is unclear.

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

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

Results: A total of 143 patients (79%) had MDS and 43 (19%) had CMML, including 108 (49%) with lower-risk based on IPSS and 114 (51%) with higher-risk disease. Therapy consisted in azacitidine monotherapy in 60 (27%) patients, decitabine monotherapy in 57 (26%), guadecitabine in 46 (21) and combinations in 59 (27%). The ORR was 61% (135/222) with 80 (36%) patients achieving CR. A total of 161 (73%) patients had at least one detectable mutation. Median number of mutations was 1 (range 0-5). Frequencies of detected mutations are shown in Figure 1A. Among 33 evaluable MDS pts, overall response by IWG criteria was 76%: complete remission (CR) in 30 (9%), partial remission (PR) in 15 (45%), and stable disease (SD) in 4 (12%).

Correlative studies suggest that RIG has chromatin modifying effects in combination with AHR2 which may overcome clinical AHR2 resistance (Chaurasia EHA 2017). Median duration of CR was 8 months for the combination. Median time to initial response was 2 cycles, and median time to best response was 3 cycles. The most frequently reported AEs were diarrhea (70%), nausea (50%), constipation (40%), fatigue (40%), and peripheral edema (40%).

Summary/Conclusions: The combination of oral RIG and standard-dose AHR2 was well tolerated in repetitive cycles in pts with AHR2 and MDS. Response was observed both in MDS treated-naive pts (85%) and in pts failing HMA therapy (62%).

Table 1.

Not available.

Figure 1.

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 MYELODYSPLOSIC SYNDROMES

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Background: As key factors in gene post-transcriptional regulation, microR-NAs (miRNAs) have been identified to play important roles in carcinogenesis in various tumors. Myelodysplastic syndrome (MDS) is a group of clonal myeloid disorders characterized by refractory quantitative and qualitative abnormalities of hemocytes and its pathogenesis is poorly understood. Some studies have shown that abnormal expressions of some miRNAs have close relationship with the pathogenesis of MDS. Recently, low RPS14 expression is found common in all kinds of myelodysplastic syndromes including patients without 5q deletion, but its mechanism remains unclear.

Aims: To determine the cause of RPS14 reduction in MDS except 5q- syndrome, influence of miRNAs on RPS14 expression was analyzed, and the role of specific miRNA on proliferation, differentiation and apoptosis of hematopoietic stem cells were evaluated. This research will help reveal the pathogenesis of MDS from a new angle and provide new ideas for the diagnosis, treatment and prognosis evaluation of MDS.

Methods: Firstly, we predicted that miR-223 may target 3’UTR of RPS14 by bioinformatics software, then verified if the special miRNA could target RPS14 by assay of luciferase activity. Secondly, the miRNA expression level of miR-223 were detected in the bone marrow BM selected from 28 MDS patients including ten RCUD patients, four RAGM-1 patients and four RAEB-2 patients, meanwhile, the miR-223 expression status were tested in four kinds cell lines including SKM-1, HL-60 /K562 and THP-1 cells lines through qRT-PCR and RPS14 expression was detected by means of immunofluorescence (IF).

Thirdly, constructing lentivirus which carried miR-223 overexpression vector and RPS14 expression was detected by means of immunofluorescence(IF). We used to induce erythroid differentiation of K562 cells by constructing lentivirus which carried miR-223 overexpression vector and RPS14 expression was detected by means of immunofluorescence(IF). We used to induce erythroid differentiation of K562 cells which carried miR-223 overexpression. We used flow cytometry method CD71 and CD235a makers and qRT-PCR (CD235 and r-globin) to detect the erythroid proliferation.

Results: We verified miR-223 could target RPS14 by assay of luciferase activity. 2. MDS patients had higher miR-232 expression than other types of MDS. Meanwhile, in cell lines, K562 cell line showed the highest level of RPS14, the miRNA expression level of miR-232 were detected in the bone marrow BM selected from 28 MDS patients including ten RCUD patients, four RAGM-1 patients and four RAEB-2 patients, meanwhile, the miR-223 expression status were tested in four kinds cell lines including SKM-1, HL-60/K562 and THP-1 cell lines through qRT-PCR and RPS14 expression was detected by means of immunofluorescence (IF).

Summary/Conclusions: MDS patients had higher miR-223 expression compared with health controls. We demonstrated that miR-223 could promote cell proliferation, inhibit cell apoptosis and suppress terminal erythropoiesis through target RPS14.

Stem cell transplantation - Clinical 1

SERIAL SEQUENCING REVEALS CLONAL ORIGINS AND STRATEGIES FOR EARLY DETECTION OF POST-ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HCT) RELAPSE IN ACUTE MYELOID LEUKEMIA (AML)

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Background: Clinical applications of next generation sequencing (NGS) in allogeneic hematopoietic stem cell transplantation are a topic of interest. Mutation dynamics post-HCT using longitudinal NGS have not been thoroughly examined. We hypothesized that serial sequencing of post-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 current 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 samples (n=57) were also sequenced. All computational and statistical analyses were performed using Python and R.

Results: The mean on-target coverage in 419 samples was 1773.7x. In total, we detected 217 mutations throughout the course of treatment in 79/88 patients (89.8%). NPM1 (26.1%), DNMT3A (26.1%), CEBPA (13.6%), IDH2 (13.6%), FLT3 (12.5%), and TP53 (11.4%) were commonly mutated at diagnosis. Unsurprisingly, most mutations appeared at initial diagnosis (200/217, 92.1%). Only 1, 2, and 14 mutations were acquired/selected at pre-HCT (0.5%), day 21 (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 selected at relapse (6.5%). 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 selected at relapse (6.5%).
serial samples, we inferred the clonal relationships between original and relapsed cGVHD 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 intermediate, containing AML and cGVHD somatic mutations 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=0.003) (Fig C-D). Multivariate analyses verified that VAF ≥0.2% at day 21 (and ≥0.1% at day 1) was associated with higher GVHD. A female donor into male recipient was associated with lower LFS, 4.62; p<0.01). Incremental age was independently associated to lower LFS, OS, GRFS and higher NRM and cGVHD. MAC was associated with lower RI (HR 1.32; 95% CI 1.01-1.72; p<0.05) and higher NRM (HR 3.03; 95% CI 1.98-4.72; p<0.01). The aim of the study was to compare outcomes of non T cell-depleted HAPLO HSCT to those from MSD HSCT. Transplantations were performed in 116 patients (49% in non T cell-depleted HAPLO recipients, 74% received PT CY 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 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. Two years, NRM and RI were 28% vs 10% (p=0.01) and 17% vs 20% (p=0.52) in HAPLO and MSD pts, respectively. Incremental age was independently associated to lower LFS, OS, GRFS and higher NRM and cGVHD. MAC was associated with lower RI and higher GVHD. A female donor into male recipient was associated to higher GVHD and lower GRFS. A longer interval from diagnosis to HSCT was associated with lower NRM and cGVHD.
ciliated to lower LFS. Donor CMV seropositivity was associated with lower GRFS and higher NRM and aGVHD. In high risk-AML aGVHD and cGVHD were 36% vs 24% (p=0.03) and 39% vs 33% (p=0.80) for HAPLO and MSD pts, respectively. At two years, NRM and RI were 18% vs 10% (p=0.16) and 21% vs 36% (p=0.02) while LFS and OS were 61% vs 55% (p=0.14) and 67% vs 66% (p=0.26) in HAPLO and MSD pts; GRFS was 49% vs 40% (p=0.17). In multivariate analysis risk of grade II-IV aGVHD (HR: 2.20; 95% CI: 1.29-3.74; 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 were associated with lower RI, higher LFS and OS. Results were confirmed in the analysis using the propensity score technique as well as for RI, NRM, LFS, OS and GRFS.

Summary/Conclusions: As per our registry based study in intermediate risk AML results of HSCT from matched sibling donor are superior to those of HAPLO-HSCT, while in high risk-AML relapse is lower in the HAPLO transplants and NRM, LFS and OS is similar.

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IMPACT OF POST-TRANSPLANT INFUSION OF DONOR T CELLS GENETICALLY MODIFIED WITH INDUCTIBLE 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 depletions reduce the risk of GVHD, but lead to delayed immune reconstitution, predisposing to serious infection and leukemia relapse due to the lack of a T-cell mediated graft-versus-leukemia (GvL). To address these challenges, we have infused mature BPX-501 T cells (donor peripheral lymphocytes which have been modified with the iCas9 suicide gene) after αβ T-cell depleted haplo HSCT to facilitate immune reconstitution and GvL effect. BPX-501 T-cells are genetically modified with the iCas9 suicide safety switch and a truncated CD19 marker. In the event of GVHD, the switch is activated by an infusion of the drug rimiducid (AP1903) resulting in rapid T cell apoptosis and GVHD reversal.

Aims: This study was performed to evaluate both safety and efficacy of BPX-501 T cell infusion post αβ T-cell depleted haplo HSCT in pediatric patients with high risk ALL and AML in CR1 and CR2.

Methods: A prospective Phase II-II study enrolling children with hematopoietic disorder who lack a matched donor. 38 patients were 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 (Bertaina, 2015 ASH). The cumulative incidence of relapse was 12.0% for BPX-501, 32.3% for MR, 22.2% for MUDs and 21.9% Haplo-αβ. Disease-free survival in the BPX-501 treated patients was 84.2% compared to 65.4% for MR, 66.1% for MUDs and 73.1% for Haplo-αβ. However, length of follow-up on the control cohorts differed from that of BPX-501 treated patients. These data suggest that BPX-501 T cells modified with the iCas9 safety switch, infused after selective αβ T-cell depletions, 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.

Figures.
Bone marrow failure and PNH

S496

HEREDITARY HEMATOLOGIC MALIGNANCIES: GENETIC COUNSELING IMPLEMENTATION IN A LARGE LEUKAEMIA 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’s goal is to apply both clinical and research testing for patients with hematologic malignancies suspected to have inherited predisposition syndromes.

Methods: Individuals were referred to the HHMC for several indications: (1) bone marrow failure/aplastic anemia/hypocellular MDS, (2) personal history of hematologic malignancy with ≥1 first-degree relative or ≥2 second-degree relatives with hematologic malignancy, (3) personal history of multiple primary cancers, (4) germline evaluation of presumed somatic mutations identified on next-generation leukemia prognostic panels, (5) management and/or surveillance of a previously-identified genetic syndrome, or (6) solid tumor hereditary syndromes. Clinical evaluation of patients 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 DiGeorge/Anderson syndrome and 3 (2%) with KS with or without leukemia. The distribution by genetic subtypes and the incidence of secondary leukemia accounts for more than 10 percent. Globinuria, abdominal pain, dyspnea, anemia (hemoglobin <100 g/L), major

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%)</td>
</tr>
<tr>
<td>ELANE</td>
<td>118</td>
<td>17 (14%)</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>SLCA6A4</td>
<td>28</td>
<td>1 (5.6)</td>
</tr>
<tr>
<td>WAS</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>JAGN1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>gene mutations without leukemia*</td>
<td>36</td>
<td>16 (11.8)</td>
</tr>
<tr>
<td>unclassified</td>
<td>30</td>
<td>16 (11.8)</td>
</tr>
<tr>
<td>Total CN</td>
<td>91</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>Elane CN</td>
<td>49</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>unclassified</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

*Gene mutations without leukemia: (G6PC3-n=9, TAZ1-n=5, p14-n=4, digenic mutations-n=4, CDH1-n=4, CDR4-n=3, germ line extracellular CS3FR-n=2, C16orf57-n=2, Pearson syndrome n=2, LYST n=1)

All subgroups benefit from G-CSF treatment. However, patients requiring maintenance doses of G-CSF above 8μg/kg/day are at greater risk of leukemic transformation.

Summary/Conclusions: Conclusion: The incidence of secondary AML reflects the genetic heterogeneity of CN.

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SECONDARY LEUKEMIAS IN GENETIC SUBTYPES OF CONGENITAL NEUTROPENIA (ELANE, HAX1, WASP, G6PC3, ETC.): A LONG-TERM ANALYSIS OF THE SCNIR EUROPE

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Background: Leukemia predisposition is well known in congenital neutropenia (CN) subtypes. By taking all patients with known and unclassified CN together the incidence of secondary leukemia accounts for more than 10 percent. Advanced molecular diagnostics and the identification of inherited and acquired gene mutations have improved our understanding of leukemic transformation in CN patients.

Aims: In the European SCNIR 449 patients with congenital neutropenia and 91 patients with cyclic neutropenia (CyN) have been enrolled since 1994. These 449 patients were evaluated by causal and follow-up. Outcomes include changes from baseline to last follow-up in LDH ratio, GPI-deficient granulocytes, red blood cell transfusions received, MAVE, and Functional Assessment of Chronic Illness Therapy (FAC-IT)-Fatigue score in patients with at least 6 months of follow-up.

Methods: Here we report the leukemia incidence of genetic subtypes analyzing all available long-term data from the European Branchof 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 transformation. The time between first detection of CSF3R mutations and onset of malignant transformation is highly variable. Some patients progressed to MDS/AML within a few months. In others, CSF3R mutant clones persisted for many years without progression to leukemia. The distribution by genetic subtypes and the frequency of CSF3R mutations is shown in the table below.

Table 1.
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=775; HDA/never euc-treated, n=636; no-HDA/euc-treated, n=111; no-HDA/never euc-treated, n=1138). Median (min, max) duration of follow-up after baseline was longer for the euc-treated patients compared with the never euc-treated patients for both the HDA and no-HDA groups (see Table). Results for changes from baseline to last follow-up in outcomes of interest are summarized in the Table. Data show that patients in the euc-treated cohort had high burden of disease at baseline. Specifically, in the HDA population, a higher proportion of euc-treated patients had a history of MAVE (33.3%) vs never euc-treated patients (13.7%). A similar disparity at baseline was also observed in the no-HDA population (33.0% vs 11.0%, respectively). Following euc treatment, the divergence in the proportion of patients with MAVE has substantially narrowed for the HDA patients (3.9% for euc-treated vs 3.3% for never euc-treated) despite longer follow-up for the treated patients. Similar findings were seen in no-HDA patients (5.3% vs 2.1%, respectively). In patients with a history of MAVE, treatment with euc was associated with meaningful improvements in mean (standard deviation [SD]) reduction from baseline in LDH ratio (-5.0 [3.7] vs -0.4 [2.3]) and proportion of red blood cell transfusion-free patients (37.6% vs 15.8%). The FACIT-Fatigue data, while limited, showed the HDA/euc-treated group experienced a greater mean (SD) score improvement than the HDA/never euc-treated group (4.1 [10.3] vs 0.5 [6.8] points).

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.

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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 can be a contributing factor in multiple hematologic malignancies, including congenital amegakaryocytic thrombocytopenia (CAMP). CAMP is a rare inherited syndrome characterized by thrombocytopenia at birth, progressing to bone marrow failure and pancytopenia. The functional impact of CAMP mutations on Mpl is yet to be determined. Here we report unique familial cases of CAMP presenting with a previously unreported MPL mutation: T814C (W272R) in the background of the activating MPL G117T (K39N or Baltimore) mutation. Aim: To determine the functionality characterization of this novel MPL mutant and the use of genome editing as a novel therapeutic option for CAMP.

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

Results: Consanguineous parents and their eldest daughter, all heterozygous for Mpl K39N/W272R, do not present any signs of disease. Their monozygotic twin daughters presented at birth with severe thrombocytopenia leading to a diagnosis of CAMP type II. Whole blood sequencing revealed a novel homozygous double Mpl K39N/W272R mutation, as their younger male sibling. One of the twins died after bone marrow transplant. Confocal microscopy shows that a significant fraction of chimeric WT Mpl protein reaches the cell surface. Significant surface expression is also noted for Mpl K39N. In contrast, the chimeric Mpl protein bearing the W272R mutation, alone or together with the K39N mutation, showed no detectable surface expression of the Tpo receptor while being strongly co-localized with ER marker calreticulin. Both WT and K39N-mutated Mpl were found signifying competent, while single or double mutants bearing W272R were unresponsive to Tpo. Tpo-induced signaling was partially rescued in GRASP55-overexpressing cells (forcing orientation of Mpl at the cell surface). Genome editing performed on cells carrying the W272R mutation restored the WT sequence and the response to Tpo, with similar cell proliferation as WT Mpl cells. Finally, when applied to primary Mpl K39N/W272R CD34+ cells, CRISPR-based gene editing rescued surface expression of Mpl and response to Tpo, as assessed by flow cytometry. K39N/W272R CD34+ cells were able to generate a similar number of megakaryocytic colonies as control CD34+ cells in a single colony assay. Non-edited cells failed to do so.

Summary/Conclusions: We report a novel double in cis mutation of Mpl (K39N/W272R) in the context of CAMP. Function of the deficient Mpl receptor could be rescued using two separate approaches: GRASP55 overexpression and CRISPR-Cas9 genome engineering. Successful editing of primary hematopoietic stem cells indicates direct therapeutic applications for gene editing in this disease.
Quality of life, palliative care, ethics and health economics

SS501

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 used at baseline, after 3 and 9 induction cycles (3ID and 9ID) and after 6 and 12 months of maintenance therapy (6MT and 12MT). The subscales global QoL, physical functioning, pain, fatigue, constipation, diarrhea, nausea/vomiting, insomnia, disease symptoms, side effects of treatment and neuropathy were analysed. Change in HRQoL score over time between treatment arms was assessed by linear mixed models. Independent sample t-tests were used to determine differences from baseline. Minimal important difference (MID) within arms was defined as a difference in score of ±1 standard error of measurement (SEM) or, if a subscale consisted of one parameter only, MID-level. Levels were used to determine clinically relevant superiority of one arm, a difference in score of ±5 was used and in addition significance level was calculated.

Results: From 553 (90.2%) of the 613 patients who participated in the HRQoL part of the study a baseline questionnaire was available. Forty (15%) of patients randomized to MPT-T versus 88 (24%) of patients randomized to MPR-R completed the therapy until 12 months of maintenance therapy. Change in HRQoL between arms over time: in MPT-T improvement of HRQoL over time as compared to MPR-R was found for the subscales diarrhea and insomnia. In contrast, MPR-R showed improvement over time for the subscales pain, constipation, nausea/vomiting, fatigue, neuropathy, and compared to MPT-T, Change in HRQoL per arm: In MTP-T MID was reached for the following subscales: global QoL increased after 9ID until 12MT (MID range 7-13), pain decreased at every time point (MID range -21 to -23), disease symptoms decreased after 9ID (MID -12), fatigue decreased during MT (MID 12) and insomnia decreased at each time point (MID range 11-23). In MPR-R the MID was reached for the following subscales: global QoL increased after 9ID until 12MT (MID range 8-14), physical functioning increased at 12MT (MID 13), pain decreased at every time point (MID range -14 to -26) and insomnia decreased at 6MT (MID -10). Difference between MPT-T and MPR-R: In the MPT-T arm significantly (p<0.05) and/or clinically (mean score difference (MSD) ≥5 points) less pain and disease symptoms at 3ID, less fatigue at 3ID and 9ID, less diarrhea and less insomnia at all time points were observed. In contrast, patients on MPR-R reported better global QoL, better physical functioning and less pain at 12MT, in general less side effects of treatment, and less constipation and neuropathy separately, at all time points than patients treated with MPT-T.

Summary/Conclusions: Both treatment with MPT-T and MPR-R controlled pain and resulted in an improvement in global QoL, as compared to baseline after 9ID and during maintenance. Treatment with thalidomide initially resulted in less pain and disease symptoms. At all treatment stages thalidomide caused less diarrhea, fatigue and insomnia as compared to treatment with lenalidomide. In contrast, therapy with lenalidomide resulted in less side effects of treatment, less constipation and less neuropathy as compared to thalidomide at all stages of treatment. In addition, long term maintenance therapy with lenalidomide resulted in better global QoL, better physical functioning and less pain.

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HEALTH-RELATED QUALITY OF LIFE RESULTS FROM THE PHASE III GALLIUM STUDY OF OBINUTUZUMAB-BASED AND RITUXIMAB-BASED THERAPY IN PATIENTS WITH PREVIOUSLYuntREATED ADVANCED INDOLENT NON-HODGKIN LYMPHOMA


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Background: Maintenance of pretreatment health-related quality of life (HRQoL) and/or meaningful improvements in HRQoL are important for previously untreated indolent non-Hodgkin lymphoma (INHL) patients (pts). GALLI-

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UM (NCT01332698) is an open-label, randomized Phase III study of obinutuzumab (GA101; G) plus chemotherapy (chemo) followed by G maintenance (G-chemo) compared with rituximab (R) plus chemo followed by R maintenance (R-chemo) in pts with previously untreated INHL. In GALLIUM, G-chemo produced a clinically meaningful improvement in investigator-assessed progression-free survival (PFS) among follicular lymphoma (FL) pts (34% reduction in risk of a PFS event relative to R-chemo). Grade 3–5 and serious adverse events were more common with G-chemo.

Aims: To compare changes in HRQoL in FL pts receiving G-chemo and R-chemo during GALLIUM.

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 well-being, and disease- and treatment-related symptoms. FACT-Lym was administered on D1 of C1 and C3 during induction, at the end of induction, and at mo 2 and 12 during maintenance/ follow-up. For each FACT-Lym scale, mean and 95% confidence interval (CI) were derived for recorded scores at each visit and changes from baseline. Minimally important differences (MIDs) were used to calculate the proportion of pts reporting improvement on the FACT-Lym lymphoma subscale (LYMs; ≥3 points), Trial Outcome Index (TOI; ≥6 points), and lymphoma total score (Lym-Tot; ≥7 points). All pts gave informed consent.

Results: Of 1202 FL pts randomized (median age, 59 yrs; 53.2% female; median observation time, 34.5 mo [range 0-54.3]), 560/601 (92.5% G-chemo) and 550/601 (91.5% R-chemo) completed all FACT-Lym scales at baseline. Baseline demographics and disease characteristics were balanced between arms. At baseline, mean HRQoL scores were similar in the two treatment arms, with all pts having some impairment of physical function, functional wellbeing, emotional and social function. Over the course of treatment, mean HRQoL was similar in the two treatment arms. From end of induction onwards, pts in both 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. There were no clear differences between arms in HRQoL scores over the course of therapy.

Summary/Conclusions: In previously untreated FL pts in GALLIUM, G-chemo and R-chemo produced similar improvements in HRQoL. These results suggest that lymphoma-related symptoms were reduced by both treatments and that the resulting improvements in well-being were not abrogated by treatment-related side effects. When viewed in the context of longer PFS, these results further support the relative benefit of G-chemo over R-chemo in GALLIUM.

**S504**

**FRONT-LINE VASCULAR ACCESS DEVICES IN ACUTE LEUKEMIAS-PEERINERALLY INSERTED CENTRAL CATHETER (PICC) VERSUS TRADITIONAL CENTRAL VENOUS CATHETER (CVC): A PHASE IV RANDOMIZED TRIAL (NCT02405728)**

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**Figure 1.**

Background: Cancer patient support groups appear to provide an important source of support to many patients and carers. In recent years there has been an increasing focus in the UK for services to provide cancer support groups, however it is unclear what proportion of patients believe access to these support groups would improve their experience of living with and beyond cancer.

Aims: A patient experience survey was undertaken by the Haematology-Oncology GMC PB across 10 NHS hospital trusts, where there are a number of cancer support groups.

Methods: The sample for the survey included all adult (aged >16) patients with a confirmed diagnosis of a haematological cancer who attended a haematological oncology outpatient appointment during a 4 month period (June-Sep-tember 2016). The survey was available for completion up to 3 months after the last follow-up contact and was completed anonymously. A translation/interpretation facility was not provided for patients whose first language was not English (due to funding restraints).

Results: 277 responses were returned with 1 response excluded (non-haematological malignancy). Haematological diagnoses included acute leukaemia (n=40), chronic leukaemia (n=35), lymphoma (n=62), myeloma (n=102), MDS (n=15), MPD (n=12), other (n=2) and not specified (n=7). 257 (93.1%) patients had received anticancer therapy, 218 (78%) were receiving treatment at the time of survey and 54% had ongoing symptoms related to their treatment or cancer. 197 (71.4%) patients did not want access to a support group, 23 (19%) wanted access, 51 (8.3%) were not aware of the possibility and 6 (1.8%) did not respond. 51.8% of patients were aware of the existing support groups, 38.8% were not sure, 2.9% were not aware and 1.8% did not respond. The cohort of patients who did or did not want access to a support group was another 8.8% and 91% respectively. 88% of patients had been given a key worker and this was particularly valuable to them. Only 20% of patients currently on treatment wanted access to a support group and 24% not on treatment wanted access to a support group. Date of diagnosis was divided into three groups. Gp A: before 2005 (n=15), Gp B: after 2006 (n=229) and not stated (n=14).

There was no difference in the three groups when asked if they wanted access to support group (13%, 22%, 7% respectively; p=0.3) or awareness that support group was available (40%, 57%, 50% respectively; p=0.6). There were additional comments from patients that support from family and online forums in addition to key workers was extremely valuable to them. On univariate analysis patients who were satisfied with their key worker support did not want access to a support group (p=0.04). There was no effect on wanting access to a support group and diagnosis (p=0.67), need for in-patient treatment (p=0.3), quality of care (p=0.8) or satisfaction with overall care (p=0.8).

Summary/Conclusions: Our results suggest that a large majority of patients with haematological malignancy do not want access to a cancer support group but providing satisfactory support through key workers and other health care professionals is likely to achieve better patient experiences.

Acknowledgements: We would like to acknowledge the members of the GMC PB and patients for their contribution to the survey.
Methods: From April 2015 to February 2017, 152 consecutive patients with acute leukemia planned for remission induction chemotherapy were randomly assigned (1:1) to PICC (Arm A) or traditional CVC (Arm B) (Table 1). Inclusion criteria were age >18 years, expected survival >4 weeks, and need of central venous access (long-term >4 weeks). Exclusion criteria were ongoing uncontrolled systemic infection, presence of significant thrombosis/stenosis in arm or central veins, and inability to communicate and/or to sign informed consent. All insertions were followed by ultrasonography assessments and chest X-ray.

Results: 152 patients (130 AML and 22 ALL) with a median age of 47 years (range, 13-82), were randomized in the two arms. In the Arm A, 76 PICCs (power injectable PICCs, in new generation polyurethane, open-ended) were inserted in 76 patients, single lumen PICCs (4 Fr) were inserted in 7 patients, and triple lumen PICC (6 Fr) was inserted in 1 patient. 68 PICCs were inserted in the right basilica vein, 5 PICCs were inserted in the left basilica vein and 3 PICCs were inserted in the left brachial vein. In Arm B, 76 traditional CVCs (untunneled heparin-coated Vialon CVC, Becton-Dickinson) were inserted by the Seldinger technique in other 76 patients. 45 CVCs were inserted in subclavian vein and 31 CVCs were inserted in internal jugular vein. Overall, the median duration of in situ catheter placement was 5 months: 6 months (range, 3-12) in the Arm A vs. 3 months (range, 1-10) in the Arm B. In the Arm A, catheter-related thrombosis occurred in 8 patients (6 basilica veins, 2 brachial veins) and catheter-related bloodstream infections in 4 patients (4 coagulase-negative staphylococci, of them, 2 meticillin-resistants). In the Arm B, 20 cases of catheter-related thrombosis (7 subclavian veins, 13 internal jugular veins) and 15 cases of catheter-related bloodstream infections (10 enterobacteriaceae; 5 coagulase-negative staphylococci and, of them, 3 meticillin-resistants) were observed. Thus, PICCs were significantly associated with fewer major complications than traditional CVCs (catheter-related thrombosis: 10.5% in the Arm A vs. 26% in the Arm B, p=0.01 by x2 test, catheter-related bloodstream infections: 5% in the Arm A vs. 19% in the Arm B, p=0.007 by x2 test) (Figure 1). Questionnaire covering activities of daily living confirmed improvement of quality of life.

Figure 1.

Summary/Conclusions: The preliminary observations of this ongoing Phase IV randomized study, focusing on front-line use of central venous access device in a high risk hematological population, suggest that the use of PICC represents an advance in terms of decrease of complication rate and improvement of quality of life for patients with acute leukemia.

SS05

THE SIMM STUDY: SURVEY OF INTEGRATIVE MEDICINE IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Pharmacologic therapy stabilizes hematologic counts and splenomegaly in myeloproliferative neoplasms (MPN), however only partial symptom improvement typically occurs. Evidence-based integrative care may address this need, however data is limited in patients with MPNs.

Aims: To investigate the association with symptom burden, quality of life, depression, and fatigue in MPN patients.

Methods: Patients were recruited via social media. Informed consent and online self-report surveys (Qualtrics) were completed, capturing patient demographics, disease specific data, supportive care utilization, MPN symptom burden (MPN-SAF TSS), depression via Patient Health Questionnaire (PHQ)-9, fatigue via Brief Fatigue Inventory (BFI) Usual, and overall quality of life (QOL) single question assessment. ANOVA, chi square tests, and Wilcoxon rank sum tests methods were applied.

Results: Patients: A total of 1087 patient surveys were consented. Of these, 885 had 10 or more responses. There were 338 essential thrombocytosis (ET), 188 myelofibrosis (MF), 315 polycythemia vera (PV), and 17 other. In MF: DIPSS risk categories included low (8%), Int-1 (19%), Int-2 (29%), high (12%), and unknown (32%). Symptom association: Overall, patients had lower MPN related symptoms when participating in aerobic activity (p<0.001), massage (p<0.001), yoga (p=0.02), strength training (p<0.001), breathing exercises (p<0.001), and support groups (p<0.001). Overall quality of life was higher with aerobic activity (p<0.001), massage (p=0.02), yoga (p=0.02), strength training (p<0.001), breathing exercises (p=0.01), and support groups (p=0.001). Depression (PHQ-9 total >3 category) was lower in aerobic activity group (p=0.001), yoga (p=0.001), strength training (p=0.001), and meditation (p=0.2). Fatigue was lower in aerobic activity (p<0.001), massage (p=0.04), strength training (p<0.001), breathing exercises (p<0.001), and support groups (p=0.001). In subgroup analysis, ET and PV patients had lower symptom burden (MPN-SAF TSS) with aerobic activity (p<0.001, <0.001), massage (p=0.01, 0.02), and strength training (p=0.03, 0.02). Support groups were found to be associated with lower symptoms in ET patients (p=0.03). In MPN, breathing exercises (p<0.001) and support groups (p=0.03) were associated with lower symptom burden. See Table #1.

Table 1.

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.
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T CELL EXHAUSTION CHARACTERIZED BY COMPROMISED MHC CLASS I AND II RESTRICTED CYTOTOXIC ACTIVITY ASSOCIATES WITH ACUTE B LYMPHOBLASTIC LEUKEMIA RELAPSE AFTER ALLO-HSCT
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Background: B cell acute lymphoblastic leukemia (B-ALL) relapse contributes to the predominant mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). However, the mechanism of B-ALL relapse after allo-HSCT remains unknown. Eradication of leukemia in allo-HSCT settings largely relies on graft-versus-leukemia (GVL) effects mediated by donor T cells. T cell exhaustion characterized by increased expression of inhibitory receptors including PD-1 and Tim-3 and impaired function may blunt the GVL effects and was reported in acute myeloid leukemia relapse after allo-HSCT; whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT remains unknown.

Aims: To evaluate whether T cell exhaustion is involved in B-ALL relapse after allo-HSCT; and investigate the correlation of inhibitory ligands on leukemic cells, leukemic load and T cell exhaustion, as well as the impact of treatment outcome on T cell exhaustion.

Methods: Our study enrolled 18 B-ALL patients who underwent first hematologic relapse after allo-HSCT and 18 matched B-ALL patients in remission (without minimal residual disease MRD) and 14 healthy donors from April 2016 to November 2016 at the Peking University People’s Hospital, Institute of Hematology. Transplant protocol and post-transplant time were matched in relapsed patients. Post-transplant time were matched as follows: ±14 days within 12 months ±1months from 12 to 18months, ±3months from 18 to 36 months, ±12months over 3 years. Extra-medullary relapse were excluded in our study. All patients had achieved full donor chimerism before relapse or bone marrow collection. Peripheral blood (PB) were collected at the same day of bone marrow collection in relapsed patients. For patients who received induction therapy, we prospectively collected BM at least once after therapy. Sample collection was performed after patients was informed consent and approval by the institutional Human Ethics Review Committee of Peking 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 setting. 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 from the tumor site are more exhausted and 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 a comprehensive functional impairment in B-ALL relapse settings after allo-HSCT and reversal of T cell exhaustion was associated with effective anti-leukemic responses. These results also provide a foundation for the development of novel effective leukemia therapeutics, such as anti-PD-1 or PD-1L-1 therapy, by targeting T cell exhaustion

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RUXOLITINIB/NILOTINIB COTREATMENT BETTER INHIBITS LEUKEMIA-PROPAGATING CELLS IN PHILADELPHIA CHROMOSOME-POSITIVE ALL Y. Kong1,2, L.-Y. Wu1, Y. Song12, M.-M. Shi12, X.-N. Cao1, H.-Y. Zhao1, Y.-Z. Qin1, Y.-Y. Lai1, H. Jiang1, Q. Jiang1, X.-J. Huang1, Y.-Z. Qin1, Y.-Y. Lai1, H. Jiang1, Q. Jiang1, X.-J. Huang1
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Background: BCR/ABL expressing cells using a qRT-PCR assay and HE and IHC with anti-hcD19 staining. Moreover, the combination of nilotinib and ruxolitinib more effectively reduced the LPCs capacity through a deeper suppression of phospho-CrKL, JAK2 and STAT5 activities at the molecular level.

Summary/Conclusions: JAK2 was more highly expressed in the sorted LPCs than in other cells of 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 optimal treatment combination was determined by the engraftment analysis of BCR/ABL expressing cells using a qRT-PCR assay and HE and IHC with anti-hcD19 staining. Moreover, the combination of nilotinib and ruxolitinib more effectively reduced the LPCs capacity through a deeper suppression of phospho-CrKL, JAK2 and STAT5 activities at the molecular level.

P508
PREDICTING ANTI-LEUKEMIA ACTIVITY OF THE BCL-2-SELECTIVE INHIBITOR ABT-199 IN BCP-ALL BY FUNCTIONAL ASSESSMENT OF APOPTOSIS SIGNALING
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Background: Although survival rates of pediatric BCP-ALL patients have continuously improved during the past decades, therapy-related toxicity and relapse occurring in 10-20 % of patients are associated with poor outcome, clearly emphasizing the need of novel, targeted treatment strategies. Deregulated survival pathways and cell death resistance contribute to treatment failure and recurrence of the disease. ABT-199 (venetoclax) is a small molecule inhibitor of BCL-2 demonstrating anti-cancer activity among different malignancies. However, predictive biomarkers are required for up-front identification of patients who would benefit from BCL-2 directed therapies.

Aims: The aims of this study were to assess the efficacy of ABT-199 in BCP-ALL, to functionally evaluate factors mediating ABT-199 susceptibility or resistance and to identify markers indicative of successful anti-leukemia activity.

Methods: The activity of ABT-199 was assessed by cell viability assays in BCP-ALL cell lines (N=6) and patient-derived xenograft (pdx) samples (N=27), analyzing half maximal effective concentrations (EC50). Expression of apoptosis regulators was detected by western blot analysis. MCL-1 deficient cell lines were generated by CRISPR/Cas9 gene editing. BH3 profiling was used to measure the mitochondrial dependence of leukemia cells on anti-apoptotic BCL-2 family proteins. In vivo treatment of ABT-199 was performed in a set of three distinct ALL rpdx.

Results: Different sensitivities of ABT-199 were observed in a series of BCP-ALL pdx and cell lines with heterogeneous anti-leukemia activities upon drug exposure. The majority of BCP-ALL samples showed sensitivity to ABT-199-induced cell death in the nanomolar range (EC50 <1µM) with four out of six cell lines and 20 of 27 pdx, while ABT-199 insensitivity with EC50s of more than 1µM were identified in 26% of pdx leukemias. ABT-199 induces apoptosis based on BCL-2 dependent sub-µM IC50. However, ABT-199 can inhibit pro-apoptotic molecules; however, the sequestration of drug-released BIM by anti-apoptotic MCL-1 might lead to resistance. Therefore, we investigated protein expression of both regulators and found the ratio (BCL-2/MCL-1) to be cor-

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related with ABT-199 sensitivity (k = 0.71, p = 0.008), highlighting the importance of expression assessment of the direct target molecule and additional resistance mediating molecules. In line, MCL-1 knockdown 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 in vivo, using a multiplexed profiling. Mitochondrial dependence on BCL-2 (multiplexed priming by the BAD-peptide measuring BCL-2, BCL-XL and BCL-W, and subtracting the response to the HRK-peptide measuring BCL-XL) was found to be tightly correlated with ABT-199 sensitivity. In contrast, ABT-199-resistant samples were characterized by low BCL-2-dependence and addition to other BCL-2 family members, including 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 = 0.14).

Summary/Conclusions: SCP-ALL displays heterogenous 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 pxd 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.

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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-leukemic cell purifications were performed by immunomagnetics methods and DNA extracted afterward. TCR-gamma gene rearrangement was studied in T- and B-leukemic cells independently by PCR sequencing. 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 on chest x-ray and TCR-gamma rearrangement was detected in purified (>95% pure) T-ALL and B-ALL cells, suggesting a common origin for both leukemic subpopulations. The B-ALL cells presented a c.35G>A p.G12D mutation in the KRAS gene, absent in the T-ALL. The T-ALL cells presented a c.35G>A (p.G12D) mutation in the NRAS gene, absent in the B-ALL. A c.1126_1127insTAGA (p.P376fs*10) mutation in the WT1 gene was also detected only in the T-ALL. A c.348+5T>(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-GEMM) presented the p.K28N mutation in the H3F3A gene.

Summary/Conclusions: Our results indicate the involvement of a Common Lymphoid Progenitor as the cell origin of this case of biallelic 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. Identification of novel biomarkers in B-cell ALL especially in those with normal cytogenetics and studying their clinical significance and biological function will be helpful for risk-stratification, treatment decision and targeted therapy.

CRSP2 (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 hepatocellular carcinoma and CSRP2 was found to be a new invadopodia bundling factor that critically promotes breast cancer cell invasion and metastasis. However, the clinical significance and biological function of CSRP2 in B-cell ALL remains unknown.
Aims: To identify novel biomarkers in B-cell ALL based on bioinformatics analyses; to examine the expression and clinical significance of CSRP2 in adults with B-ALL; to explore effects of CSRP2 on biological function of B-cell ALL.

Methods: We did bio-informatics analyses to identify mRNA transcripts aberrantly-expressed in B-cell ALL. RT-qPCR (real-time quantitative polymerase chain reaction) was used to examine CSRP2 transcript levels in bone marrow samples from 236 adults with B-cell ALL compared with samples from normal. A prognostic value was assessed in 168 subjects. CSRP2-knockdown and CSRP2-over-expression cell models were constructed to study the biological function of CSRP2 in B-cell ALL.

Results: We selected 9 candidate genes for validation 7 of which proved significantly-associated with B-cell ALL. CSRP2 was the most differentially-expressed gene in our validation studies. CSRP2 was over-expressed in 228 out of 236 adults (97%) with newly-diagnosed B-cell ALL. In subjects with normal cytogenetics: those with high CSRP2 transcript levels had a higher 5-year cumulative incidence of relapse (CIR) and worse relapse-free survival (RFS) compared with subjects with low transcript levels (56% [95% confidence interval 53.5-69%] vs 19% [18-20%]; P=0.011 and 41% [17.65%] vs 80% [86-96%]; P=0.007). In multivariate analyses a high CSRP2 transcript level was independently-associated with CIR (HR=5.32 [1.64-17.28]; P=0.005) and RFS (HR=5.56 [1.87-16.53]; P=0.002). Functional analyses indicated CSRP2 promoted cell proliferation, cell-cycle progression, in vitro colony formation and migration. Abnormal CSRP2 expression was associated with resistance to chemotherapy; sensitivity was restored by down-regulating CSRP2 expression. CSRP2 activated ERK1/2 signaling pathway, regulated cell-cycle related protein and activated CREB signaling pathway, whose activation was associated with poor prognosis in adults with B-cell ALL.

Summary: CSRP2 was widely over-expressed in adults with B-cell ALL. Determination of CSRP2 transcript levels in subjects with normal cytogenetics might inform therapy-decisions. Consideration could be given to down-regulating CSRP2 expression as a way to reverse drug resistance.

P512

THERAPEUTIC TARGETING OF PRE-B CELL RECEPTOR SIGNALLING IN CHILDHOOD ACUTE LYMPHOBластIC LEUKEMIA

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Background: Acute lymphoblastic leukaemia (ALL) is the most common malignancy in children and adolescents and relapsed ALL remains one of the leading causes of cancer-related deaths in children. Components of the precursor-B cell receptor (Pre-BCR) signalling pathway are hijacked in ALL cells and this dependence may be therapeutically targeted. A number of tyrosine kinase inhibitors (TKIs) targeting effectors of this signalling pathway are showing great promise in the clinic and warrant preclinical evaluation in paediatric ALL. They include Dasatinib (BCR-ABL/SRC inhibitor), Fostamatinib R406 (SYK inhibitor), Ibrutinib (BTK inhibitor) and CAL-101 (PI3Kδ inhibitor).

Aims: To preclinically evaluate these candidate TKIs, as novel, targeted drugs for the treatment of relapsed ALL.

Methods: ALL cell lines (Reh, Nalm-6, PreB 697 and its glucocorticoid resistant descendant, R3F9) and 36 primary-derived xenograft (PDX) cells from 16 ALL were used in the study. Cell viability was assessed by Resazurin. Pre-BCR expression (µHc, VpreB and 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-ϒ2, p-BLNK). Apoptosis and cell cycle were analysed by Annexin V and Propidium iodide. RQ-PCR was used to measure expression levels of key proteins. Cell cycle, DNA content and apoptosis were measured by DNA staining, GFP expression and phosphorylation were detected by western blotting.

Results: ALL cell lines were modestly sensitive to Dasatinib (mean G50 5.33 µM, range 2.45 µM-12.5 µM) and R406 (mean G50 4.32 µM, range 2.88 µM-5.83 µM). However, cells were resistant to Ibrutinib (mean G50 15.9 ± µM, range 11.47 µM-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 were sensitised to Ibrutinib (mean G50 15.9 ± µM, range 11.47 µM-18.3 µM) and CAL-101 (3 out of 15 patient samples <2.5 µM). Pre-BCR positive ALL cell lines and PDX cells were sensitive to R406 and Dasatinib, with a Ph+ PDX confirming sensitivity to the latter. Combining TKIs with the glucocorticoid (GC), Dexamethasone showed synergism in GS 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.
Acute lymphoblastic leukemia - Clinical 2

P515
SYSTEMATIC MRI SCREENING IDENTIFIES EXTENSIVE ASYMMETRIC OSTEOSCLEROTIC 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 prospective multicenter 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 AEIOU-BFM 2009 trial, 25 (39.1%) pts to the CoALL-08-09 trial and 3 (4.7%) pts were enrolled in the NLL-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 asymmetric osteosclerotic lesions stage II or higher in 3 of 60 pts (5%), at 6 months in 7 of 34 pts (20.6%) osteosclerotic 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 ON stage II 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 epiphyseal 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. Epiphyseal 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 symptomatic 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.
Background: Front-line imatinib (IM) plus chemotherapy followed by alloge
netic hematopoietic stem cell transplantation (HSCT) is standard therapy for patients (pts.) with Ph+ ALL. Relapse after HSCT remains a major cause of treatment failure, and pts. in whom BCR-ABL transcripts are detectable after HSCT are at particular risk. Post-transplant maintenance with low dose 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 q-RT-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 enrollment, sufficient hematopoietic and organ function, and no prior IM or infection. The dose of IM was 600/400 mg in 22% of pts., and 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 PBSC 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 600/400mg in 22% of pts., remaining pts. received 400mg. Treatment was prematurely discontinued in 56% and 59% of pts., median time to discontinuation was 575d and 594d, respectively. Target dose of 600mg (10%). Rates of G3/4 AEs and SAEs decreased substantially >8 wk post infusion. CRS was managed with supportive care, and embolic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days). Safety events were similar across pt subgroups based on age, sex, prior ALL, and embolic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days). Safety events were similar across pt subgroups based on age, sex, prior ALL, and embolic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days).

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 G TKI remain to be determined.

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ANALYSIS OF SAFETY DATA FROM 2 MULTICENTER TRIALS OF CTL019 IN PEDIATRIC AND YOUNG ADULT PATIENTS WITH RELAPSED/ REFRACTORY (R/R) B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL)


Background: The CD19-targeted chimeric antigen receptor (CAR) T-cell ther-
apy 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. This study serves to identify any new safety issues with CTL019 emerging from use in multicenter 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 (NCT024353849 and NCT02228096) were used to further characterize the safety of CTL019.

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 premedication failure). 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 at least 1 AE (57% G1, 32% G2, 9% G3, 0% G4). The most common AEs were decreased appetite (21%), increased AST (19%) and ALT (12%), hypoxia (16%), hypokalemia (13%), hypophosphatemia (11%), and pulmonary edema (10%). Rates of G3/4 AEs and SAEs decreased substantially >8 wk post infu-
sion to 24% and 12%, respectively. 10 pts died post infusion, 16 (10%) from B-ALL (n=2, ≤30 days after infusion; n=14, >30 days); cerebral hemorrhage (n=1) and embolic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days).

Safety events were similar across pt subgroups based on age, sex, prior ALL, and embolic infectious stroke (n=1) (both ≤30 days); and infection (n=3, >30 days).

Conclusions: The incidence of CRS was greater than expected based on historical experience and the CRIS registry. Treatment with this investigational therapy was well tolerated across all pt subgroups.

Table 1.
twice as likely to develop G3/4 CRS than pts with <50% BM blasts (n=29) (63% vs 24%). Earlier-onset fever correlated with severity of CRS. CRS grade correlated with serum IL-6 levels. CRS-associated coagulopathy with fibrinogen levels <1.0 g/L was observed in 10% of pts. Neuropsychiatric AEs occurred during or shortly after CRS resolution, were self-limiting, and were more likely in pts with severe CRS or history of CNS leukemia or other CNS diseases. No G4 neuropsychiatric events were observed. Other AEs of special interest within the first 8 wk included G3/4 neutropenia with high (>38.3°C) fever (61%) and infections (G3/4, 22%). Prolonged G3/4 neutropenia (not resolved >28 days) occurred in 59 pts (61%). 36% of pts with prolonged G3/4 neutropenia had G3/4 infections after day 28. One pt with prior alloSCT was diagnosed with unconfirmed gut GVHD. Responding pts developed prolonged B-cell aplasia that was managed with immunoglobulin replacement. Tumor lysis syndrome was uncommon (3%).

Summary/Conclusions: This pooled analysis of global experience with CTL019 across 25 sites and 11 countries found no new safety issues. CRS and neuropsychiatric events, which are class effects of CAR T-cell therapy, were effectively managed. CTL019 appears similarly safe in pts with Down syndrome or prior alloSCT and across age groups. Prolonged follow-up will be required to determine the long-term safety of B-cell aplasia.

**Results:**

- Ponatinib is a third-generation pan-BCR-ABL inhibitor that is effective in Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) and overcomes the T315I gatekeeper mutation.
- Aims: We designed a phase II trial to evaluate the efficacy and safety of hyper-CVAD plus ponatinib in adults with newly diagnosed Ph+ ALL.
- Methods: Adults with newly diagnosed Ph+ ALL received 8 cycles of hyper-CVAD alternating with high dose MTX/Ara-C every 21-28 days, as permitted by peripheral count recovery. Patients (pts) who were refractory to 1-2 cycles of hyper-CVAD (CD20 expression was retested) with or without other TKIs were still eligible. Ponatinib was given at 45mg daily on days 1-14 of cycle 1. Initially, pts then received ponatinib 45mg daily continuously beginning with cycle 2. Due to concern for potential cardiovascular toxicity with ponatinib, after 39 pts had been treated, the protocol was amended so that, beginning in cycle 2, pts in CR received ponatinib at a dose of 30mg daily and pts in complete molecular response (CMR) received 15mg daily. Rituximab was given during the first 4 cycles in pts with CD20 expression ≥20%; all pts received IT chemotherapy prophylaxis with the first 4 cycles. Pts in CR after 8 cycles of hyper-CVAD received maintenance with ponatinib, vincristine, and prednisone for 2 years followed by indefinite daily ponatinib.
- Results: To date, 64 pts have been treated; 63 pts have received prior treatment at the time of enrollment, 8 of whom were in CR and 2 of whom were not in CR. Median age was 48 years (range, 21-80 years); 23 pts (36%) were positive for CD20 expression. BCR-ABL1 transcript was p190 in 45 pts (70%), p210 in 18 (28%) and unknown in 1 (2%). Of 56 pts not in CR at the time of study enrollment, 100% achieved CR; all but 1 pt achieved CR after the first cycle. The complete cytogenetic response rate was 98%, major molecular response (MRM) rate was 97% and CMR rate was 77%. The median time to MMR was 3 weeks (range, 2-14 weeks), and the median time to CMR was 10 weeks (range, 2-96 weeks). The median follow-up was 33 months (range, 2-62 months), and median number of cycles received was 6 (range, 2-8 cycles). 10 pts (16%) underwent allogeneic stem cell transplantation (ASCT) in first remission. Overall, 8 pts have relapsed (3 while still on ponatinib, 3 while on another TKI, 1 while not receiving a TKI, and 1 after ASCT). 14 pts have died, 8 of whom were in CR. Two deaths were attributed to ponatinib (both from MI while in CR). 38 pts are still receiving treatment (consolidation, n=7; maintenance, n=14; post-maintenance TKI, n=17). The 3-year continuous CR and OS rates were 79% and 76%, respectively. In a 4-month landmark analysis to assess the impact of ASCT, continued CR and OS did not differ according to whether ASCT was performed in first remission. Treatment was overall well-tolerated. Myelosuppression was as expected in a CVAD-based regimen: median times to ANC and platelet recovery in cycle 1 of 22 days (range, 17-35 days) and 18 days (range, 13-29 days), respectively. In cycles 2-8, the median times to platelet and ANC recovery were 22 days and 16 days, respectively. Grade ≥3 adverse events included pancreatitis in 12 pts (19%), hypertension in 8 (13%), thrombotic events in 4 (8%) and MI in 3 (5%). No grade ≥3 cardiovascular events have occurred after 48 weeks of treatment with ponatinib.

**Summary/Conclusions:** Hyper-CVAD plus ponatinib is effective in patients with newly diagnosed Ph+ ALL, resulting in a CMR rate of 77% and encouraging long-term survival.
A PHASE II STUDY WITH A SEQUENTIAL CLOFARABINE-CYCLOPHOS- PHAMIDE COMBINATION schedULe FOR SALVATION TREATMENT FOR REFRACTORY AND RELAPSed ACUTE LYMPHOBLASTIC LEUKEMIA (R/R) IN ADULT PatIENTS

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Summary/Conclusions: Blinatumomab showed antileukemia activity in pediatric and adolescent patients with high-risk relapsed/refractory B-precursor ALL including t(17;19) and AEs were consistent with those previously reported for relapsed/refractory ALL.

Table 1.

Within the first two cycles

**Method:** Open-label, single-arm, multicenter, single-institution, global, phase IIa study for pediatric and adolescent patients with relapsed/refractory B-precursor ALL. Eligible patients (aged >28 days to <18 years) had ≥5% blasts and marrow blast percentage were 5.67% (range 1.5-25.1%), 82 patients were enrolled. Median patient age was 38.7 years (range 20.5-59.6), 15 were male, 4 with T- and 23 B-precursor ALL, 2 refractory and 25 relapsed (after a median of 5.9 months, range 1.9-23.4), 5/11 evaluable with high-risk cytoge- netics [2 complex; 2 t(4;11), 1 MLL-rearranged]. Median white blood cells and marrow blast percentage were 5.67% (range 1-55) and 73% (range 8-100), respec- tively. All but one patient (treatment interruption due to traumatic fall) received CY-CLO as planned. Nine patients achieved CR and 7 CRi after course 1 (over- all response 92.2%; 6/10 in study stage one), 2 had a partial response, 5 refractory, 3 died early and one survived with pancytopenic marrow. In relapsed patients, only the length of prior remission affected the probability of CR, from 24.4% (n=18) to 50% (n=6) if ≤ or >12 months, respectively (P=0.02). In 10 evaluable patients, median post-induction MRD was 0.01% (<0.001% in 3). Six CR patients received a second course, which was curtailed in 2 due to tox- icity, and 10 responders had an allogeneic SCT when possible.

**Background:** Blinatumomab, a bispecific T-cell engager antibody construct, has shown antileukemia activity and tolerability in patients with relapsed/refrac- tory B-precursor acute lymphoblastic leukemia (ALL).

**Aims:** We further evaluated safety and efficacy of blinatumomab in pediatric and adolescent patients with relapsed/refractory B-precursor ALL enrolled in an expanded access study (NCT02187354).

**Methods:** Eligible patients (aged >28 days to <18 years) had ≥5% blasts and relapsed/refractory B-precursor ALL (refractory to prior treatments, second or later relapse, or relapse after allogeneic hematopoietic stem cell transplantation [alloSCT]). Blinatumomab was dosed by continuous intravenous infusion (4 weeks on/2 weeks off) for up to five cycles (≥5 <25% blasts: 15µg/m²/day; ≥25% blasts: 5µg/m²/day on days 1-7 in cycle 1, then 15 µg/m²/day). The primary endpoint was incidence of treatment-emergent (TE) and treatment-related (TR) adverse events (AEs). Key efficacy endpoints were complete response and minimal residual disease (MRD, measured by polymerase chain reaction or flow cytometry) response within the first two cycles, relapse-free survival, overall survival, and incidence of alloSCT.

**Results:** Among the first 40 treated patients (median age, 9 [range, 1–17] years), 24 (60%) had experienced ≥2 relapses, 20 (50%) had relapsed after alloSCT, and 5 (13%) were primary refractory; 18 (45%) had ≥50% blasts and 21 (53%) had prior alloSCT. Safety and key efficacy outcomes are shown in the table. Twenty-five patients (63%) achieved a complete response within the first two cycles; 19 of whom had an MRD response. Eight patients relapsed and 20 died after treatment. Regardless of causality, the most frequent TEAEs were pyrexia (78%), cytokine release syndrome (CRS; 23%), vomiting (23%), and anemia (20%). All nine CRS events were grade 1 or 2, and one tumor lysis syndrome was grade 3. Ten (25%) patients interrupted treatment and two (5%) discontinued due to TRAEs; 13 (33%) patients had grade ≥3 TRAEs, including two of three neurologic events (depressed level of consciousness and headache; both grade 3). Two patients experienced fatal AEs, both of which were considered unrelated to blinatumab.
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 2–5 days. Of the 10 treated pts, 9 had high BM 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 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.

Response: 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 2–5 days. Of the 10 treated pts, 9 had high BM 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 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.

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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 versus (v) conventional chemotherapy (C) in the Phase 3 INO-VATE trial (NCT01564784; Kantarjian NEJM 2016 [data as of October 2, 2014]. More InO v C patients (pts) proceeded to hematopoietic stem cell transplantation (HSCT); 41% [45/109] v 11% [12/109]; P<0.001).

Aims: To assess factors associated with outcomes after allogeneic HSCT in patients with R/R ALL who were previously treated with InO.

Methods: Full details have been published. Informed consent was obtained from all patients. Multivariate analyses (MVA) using Cox regression modeling were conducted to determine predictors of non-relapse mortality (NRM) and overall survival (OS).

Results: As of March 8, 2016, 108,326 pts underwent allogeneic HSCT (InO n=77; C n=31). Baseline characteristics were generally similar, except baseline platelet values were lower in InO v C pts. More InO v C pts achieved minimal residual disease negativity during study therapy (MRD<0.1% [best status]; 71% v 26%; P<0.0001). Less InO v C pts received additional therapy before HSCT (14% v 55%, P<0.0001). NRM rates were higher in InO v C pts at 1 year (yr); 36% [95% CI 26–47] v 20% [8–36] and 2 yrs (39% [27–51] v 31% [13–51]), but relapse rates were lower (1 yr, 23% [15–33] v 29% [13–48]; 2 yrs, 33% [22–44] v 46% [24–65]). No significant difference in post-HSCT survival was detected in InO v C pts; however, visual inspection of the curve suggested the survival probability varied before and after 15 months post-HSCT (1 yr, 44% [95% CI 33–55] v 65% [44–79]; 2 yr, 39% [28–50] v 34% [15–54]). Fatal veno-occlusive disease (VOD) was observed in 5 InO pts (during the first 100 days from the date of HSCT) and no C pts. MVA showed that conditioning regimens without dual alkylators and thiopeta were associated (2-sided; P<0.05) with lower risk of NRM and post-HSCT survival, respectively.

Summary/Conclusions: Compared with C, InO permitted more pts with R/R ALL to proceed to HSCT in CR/CRi with MRD<0.1% (best status). Despite increased NRM and fatal VOD, long-term survival was attainable in InO pts. In pts previously treated with InO, interventions to reduce NRM and improve OS after HSCT include avoiding dual alkylator conditioning regimens, especially those containing thiopeta.
Aims: This work examines how ENL influences PRC1 repressive activity. Methods: The effect of ENL on transcriptional activity of model promoters and endogenous transcriptional control elements was studied by biochemical and molecular biology methods. Results: Here we demonstrate that ENL overcomes polycomb induced silencing through recruitment of polymerase associated factor 1 (PAF1) a chromatin remodeler. We observed an intrinsic ability to bind PAF1 and further showed that the ability of ENL to neutralize polycomb-mediated repression in an elongation reporter system and also during transformation of primary cells by MLL-ENL in vivo. Inactivation of polycomb by ENL was accompanied by ubiquitination of histone H2B, the hallmark activity of PAF1 allied enzymes. On a global scale we performed recent RNA-Seq demonstrated that MLL-ENL target genes stood out with a supraphysiological accumulation of H2BUB accompanied by hyper-accelerated transcription rates. Interestingly, examination of Wilms tumor specific ENL mutants allowed to elucidate the underlying mechanism of the MLL-fusion induced ENL hyperactivity. Introduction of Wilms-specific ENL into primary hematopoietic cells induced aberrant transcription and H2BUB modification of Hox9a and Meis1, two sentinel loci for polycomb action. This was dependent on the conserved YEATS domain of ENL that operated as “switch” binding either histone H3 or PAF1 thus effectively regulating ENL function as anti-repressor or elongator factor, respectively. Wilms tumor specific ENL interaction 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.

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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 acceleration 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, PKCg 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 PKCg and SOD2 were performed with recombinant lentiviruses expressing gene-targeting shRNAs. Recombinant retroviruses expressing either PKCg or SOD2/Catalase were used for gain-of-function assays. Cytosolic and mitochondrial redox states were measured using redox-sensitive GFP (roGFP) probes followed by flow cytometric analysis. Mitochondrial superoxides were also assessed by flow cytometric analysis of MitoSox stained cells. Proteomic analysis was achieved using nano LC-MS/MS. Annexin-V staining was analyzed by flow cytometry to measure cell death and in vitro migration assays were performed using transwell chambers. FACs-based purification of sRNA-expressing cells followed either by: 1) growth in cytokine-enriched media or 2) transfection into syngeneic mice for survival analysis.

Results: We have discovered that inhibition of PKCg: 1) promoted the death of AML cell lines in vitro, 2) reduced AML cell proliferation 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 PKCg 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 PKCg can protect AML cells from lethal effects of superoxide-inducing agents 2-thiophenyltrifluoroacetone and Antimycin A. To identify potential ROS-regulatory enzymes downstream of PKCg, we performed whole cell proteomics and found that the mitochondrial superoxide-neutralizing enzyme SOD2 is decreased in AML cells depleted of PKCg. Similar to PKCg inhibition, we also observed enhanced genetic 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 PKCg inhibition confirming that PKCg supports AML pathophysiology by maintaining mitochondrial redox homeostasis.

Summary/Conclusions: Our results indicate that PKCg 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.

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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 compared to traditional chimeric AML or FLT3 targeted kinase inhibitors.

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/FTet2F/Flt3ITD/+Mx1Cre+ or Ptnp11F/FTet2F/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 leukemia 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=8). Though the Ptnp11 deleted leukemic mice showed almost complete loss of long term HSC and concomitant increase in short term proliferating HSC in the bone marrow, they were still able to home and engraft in lethally irradiated recipient mice. These results indicate that loss of Ptnp11 does not impair the engraftment of leukemic stem cells though in normal mice deletion of Ptnp11 impaired the ability to stem cells to home to bone marrow niche and engraft. Deletion of Ptnp11 in both primary mice and secondary recipients was also associated with deregulation of myeloid and lymphoid cell distribution both in the periphery and bone marrow. Mice with deletion of Ptnp11 in the context of Flt3ITD did not generate immature or mature B cells. The effects of Ptnp11 deletion were more severe in in vivo mice 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 once observed that the ability to induce hematopoietic 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 REPRESSION OF DIVERSE ORIGINS

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gene mutations and the sensitivity of leukemic cells to kinase inhibition depends in inhibitors remains poorly defined and these have not been translated into effective treatment of AML, the high recurrence of activating mutations in kinases and other oncogenes in AML is a major challenge. The success of kinase inhibitors in leukemia treatment is based on their ability to irreversibly block the RUNX cluster expression profiling through disrupting 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 RUNX1. Analysis of these residual AML cells revealed that RUNX1 depletion induced remarkable induction of p53 as well as its target gene products and additive knockdown of p53 in these cell lines revert the phenotype of RUNX1-depletion, indicating that RUNX1 is functionally dependent upon p53 pathway. In addition, cycloheximide chase assay revealed that RUNX1 negatively regulates the protein stability of p53 in AML cells. In silico data analysis and ChIP-seq 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 in the absence of silencing of RUNX1. Analysis of these residual AML cells revealed the reciprocal up-regulation of RUNX2 and RUNX3 expressions, suggesting that RUNX2 and RUNX3 might compensate for the loss of RUNX1 functions. As expected, additional knockdown of RUNX2 and RUNX3 in RUNX1-depleted AML cells effectively suppressed their proliferations. Thus the simultaneous targeting of all RUNX cluster 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 vivo. Moreover, this reagent was exceptionally well-tolerated in mice and exhibited excellent efficacy against xenograft mice models of AML, acute lymphoblastic leukemia, lung and gastric cancers, extending their overall survival periods in vivo. Since RUNX families take part in diverse physiological functions not only in AML cells but also in normal hematopoietic cells and in various other vital organ tissues, we might expect criticisms in targeting whole RUNX family that it could trigger undesirable side-effects in vivo. Intriguingly, however, our drug was well-tolerated in vivo and through these experiments, we have coincidentally found that the amount of total RUNX expressions was consistently higher in malignant tissues compared to their normal counterparts, and we believe that this gap offers pharmacological window to be targeted in the maintenance and progression of cancer and the indicated gene switch technology-dependent its modulation would be a novel strategy to control malignancies.

Figure 1.

Methods: In this investigation, we used a multimodal approach to stratify 36 AML biopsies as a function of their cellular sensitivity to "ex vivo" treatment with TAK-715, silmitasertib, PF03758309, midostaurin and trametinib, which target P38, ATR, AKT, PAK, PKC, and MEK and ERK, respectively. The same samples were analysed using different omics platforms: (i) mass spectrometry for phosphoproteomics, proteomics and kinomic profiling, (ii) mass cytometry for immunophenotyping and (iii) next generation sequencing for mutational profiling.

Results: Our integrative analysis identified two independent signatures that stratified our cohort of patients in sets of differentiated and undifferentiated cases. The phosphoproteomics signature divided our set of AML cases in the M1-like and M4-like groups (Figure 1A). The mass cytometry signature, which represented myelomonocytic markers that were co-expressed at the cell surface, split our cohort of patient in the CD8+ and CD4+ groups. Remarkably, the M4-like and CD8+ groups representing the differentiated cases, as well as the M1-like and CD4+ groups representing the non-differentiated cases, showed a high degree of overlap. Differentiated groups over-phosphorylated 3 times as many proteins as the non-differentiated groups, including kinases at sites linked to their activity. Mutations in genes involved in kinase signalling were also more frequent in differentiated cases. Kinase activity analysis using KSEA estimated that differentiated groups presented an enriched activity for PAK, MEK, ERK or PKC. Ontology analysis showed that non-differentiated cells over-phosphorylated nuclear proteins with DNA binding properties, while the differentiated cells increased the phosphorylation of membrane and cytoplasmic proteins linked to the small GTPase signalling. More interestingly, cases in differentiated groups were more sensitive to PF03758309, trametinib and midostaurin than those in the non-differentiated sets (Figure 1B for groups defined by the phosphoproteomics signature). Finally, differentiated cases as defined by the mass cytometry signature in our cohort of patients, or by a CD marker mRNA expression signature in the ATCG database, presented with significantly reduced survival when compared to the groups of non-differentiated cases.

Summary/Conclusions: Our data indicate that differentiated cells activate pro-survival kinases like PAK, PKCD or MEK which make them more sensitive to the inhibitors PF03758309, midostaurin or the FDA-approved drug trametinib. Since patients with differentiated cells present a reduced overall survival, treatment with these compounds may benefit patients in this higher risk group.

Figure 1.
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 CCAATenhancer-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 (CEBPAmut) are observed in AML with normal karyotypes, and approximately 60% of the mutated patients (pts) carry biallelic mutations. Several studies showed that CEBPAmut occur almost mutually exclusive with regard to other AML associated gene mutations such as NPM1 or FLT3-ITD mutations. Recently, mutations in the tet oncogene family member 2 (TET2mut) were described as a frequent concurrent mutation of CEBPAmut. Both genes are involved in the control of proliferation (CEBPA, TET2) and differentiation (CEBPA) of myeloid progenitors. Preliminary data suggest that pts harboring the CEBPAmut/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 CEBPAmut AML pts.

Methods: In total 200 AML pts (age 18 to 78 years) with CEBPAmut (n=113) or CEBPA single mutations (CEBPAwsm) (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; AMLHD98B (NCT00146120) n=53; AML-98A (NCT00151242) n=74; AML-06-04 (NCT00151255) n=25 and AMLSG 12-09 (NCT01180322) n=22). TET2 mutation screening was performed using a DNA-based PCR-assy assay 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 co-occurred with CEBPAmut (16/113, 14.2%), 23 pts had concurrent CEBPAwsm (23/87, 26.4%). All TET2mut were heterozygous, with 13 pts having two mutations. The median follow-up of the 200 pts was 76.3 months (95%CI: 68.5–84.2). TET2mut were restricted to the cytogenetic intermediate-risk group (100%), and pts with TET2mut were significantly older than pts with TET2 wild-type (TET2wt) (49y vs 46y, 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, P < 0.001 and P < 0.001, respectively). Especially in pts with low-age (60y or younger) and in pts with CEBPAmut (P < 0.001), we found a significant association of TET2mut with older age (49y vs 46y, P = 0.05), and an inferior EFS (P = 0.001), RFS (P = 0.003), and OS (P = 0.07). Finally, we analysed the impact of TET2mut within the subgroup of CEBPAmut pts (n=87). In this subgroup of TET2mut we found to be significantly associated with older age (60y or younger) and with SRSF2 mutations (P = 0.02). Clinically, pts with TET2mut had a shorter RFS (P = 0.02) and OS (P = 0.05), and in trend a shorter EFS (P = 0.09).

Summary/Conclusions: In our study on a large cohort of CEBPAmutAML pts we could confirm the high incidence of concomitant TET2mut (19.5%). Pts with concurrent TET2mut were significantly older and had an inferior outcome.

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GF1B–A NOVEL ONCOSUPPRESSOR WHICH RESTRICTS NUMBER OF LEUKEMIC STEM CELLS

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Background: Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are hematopoietic disorders, which affect the myeloid lineages of hematopoiesis. Both are characterized by an accumulation of blast cells in the bone marrow (BM) that have the lost the ability to differentiate to mature cells. The proper differentiation of hematopoietic stem cells (HSCs) is regulated by transcription factors. Growth factor independence 1b (GF1B) is a repressing transcription factor regulating quiescence of HSCs and the proper emergence and maturation of erythrocytes and platelets.

Aims: Aim of the study was to identify i) do different level of GF1B influence onset and development of MDS and AML in human patients ii) how does GF1B act in MDS/AML development on a molecular level.

Methods: We correlated GF1B expression level in blast cells of patients with MDS and AML with the overall disease course. To get a better insight how does GF1B influence MDS/AML development we used three different murine models of human AML with expression of different oncogenes (NUP98/HOXD13, MLL-AF9 and expression of a mutated K-Ras). In these models we either downregulated or conditionally knocked out GF1B expression. Finally, we performed ChIP Seq analysis as well as whole genome expression arrays to study the molecular functions of GF1B in AML development.

Results: Low expression or absence of GF1B expression was associated with an inferior outcome with regard to overall-survival as well as event-free survival of MDS/AML patients. Using the above murine models of MDS/AML, loss or low expression of GF1B accelerated AML development. Additionally we could show that GF1B deficiency restricted number of functional hematopoietic stem cells. It is well known that GF1B has a function to recruit histone modifying enzymes to induce among other deacetylation of H3K9. ChIP seq data of GF1B deficient leukemic cells revealed that loss of GF1B led to a higher H3K9 acetylation of a target of target genes, among them a number of oncogenes. Among these target genes, we found MAPK as well as Reactive oxygen species (ROS) signalling, as one of the top hit in our data. Previously it was reported that loss of GF1B enhanced the ROS level in HSCs. In our case we also saw an increased expression of ROS in GF1B deficient leukemic cells, a higher activity of the FOXO pathway as well as reduced p38 activity. The combination of these findings connects the higher number of leukemic stem cells in GF1B deficient leukemic cells. To reduce the high level of ROS in leukemic stem cells we use N-Acetylcystein (NAC). Use of NAC impeded growth of GF1B deficient cells in vitro.

Summary/Conclusions: GF1B act as a tumoursuppressor by restricting number of leukemic stem cells and treatment with NAC opens a potential targeted therapy for AML patients with low/absent expression of GF1B.

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VARIANTE ALLELE FREQUENCY KINETICS OF TYROSINE KINASE GENE MUTATIONS IN CORE-BINDING FACTOR ACUTE MYELOID LEUKEMIA AT THE TIME OF DIAGNOSIS AND DURING REMISSION UNDER TREATMENT WITH DASATINIB


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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), the clinical architecture of relapsed disease is not well defined. Mutations affecting signaling genes, such as KIT and NRAS are known to be among the most common oncogenic drivers in CBF-AML, however their impact at relapse remains unclear.

Aims: To characterize clonal evolution in paired samples obtained at diagnosis, during remission and at relapse under treatment with dasatinib, and ii) to conduct gene set enrichment analyses.

Methods: Whole-exome-sequencing (WES) was performed in paired diagnosis, remission and relapse samples of 38 patients with CBF-AML (inv(16), n=24; t(8;21), n=12) using paired-end sequencing (read length 100 bp) on an Illumina HiSeq platform. Library preparation was done with the Nextera® Rapid Capture Exome kit following manufacturer’s instructions. All patients were treated within one of five trials of the German-Austrian Study Group (AMLSG). In two of the trials (AMLSG 11-08, NCT00850382; AMLSG 21-13, NCT02013648) patients received intensive chemotherapy in combination with the multi-kinase inhibitor dasatinib.

Results: The mean WES coverage was 133x. Mutations and indels were called with a threshold >10% variant allele frequency (VAF) after filtering for SNPs and sequencing artefacts. In sum, we identified 587 variants in 430 genes. At
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 mutant patient treated with dasatinib the baseline KITD185V mutation (exon 17) was lost at the time of relapse, but a KITD192Y 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 CBF-AML subtype. Our data suggest that the KIT clone might be successfully eradicated under dasatinib treatment whereas persistence of KIT mutant clones was more commonly seen under conventional chemotherapy. The frequent loss of KIT and NRAS mutations during therapy suggests that relapse is triggered by alternative genetic lesions. Relapsed disease may represent a distinct biology which is characterized by mutations that cluster in different pathways. Further analyses are ongoing including study cohort expansion, as well as inclusion of RNA sequencing results.

**P536**

**Acute myeloid leukemia - Biology 4**

**P38 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 antitumor efficacy 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 high expression of p38β protein levels 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, facilitates its trafficking to the cytoplasm, contributing to PP2A inactivation in AML cells. Moreover, p38β binds to SET in the cytoplasm, contributing to its stability and leading to PP2A inactivation. In this regard, we have preliminary evidences that combination therapy with PADs and the CK2 inhibitor CX4945 reduces significantly the viability of AML cells, supporting that novel treatment modalities that can target multiple components of the same pathway may help to achieve a more sustained therapeutic benefit.

**P537**

**GENETIC LANDSCAPE OF ACUTE ERYTHROID LEUKEMIA**

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Background: Acute erythroid leukemia (AEL) is a unique subtype of acute myeloid leukemia (AML) characterized by the predominance of erythroid components with increased ring sideroblasts as well as frequent myelodysplasia. However, due to its rarity, the molecular pathogenesis of AEL has not been fully elucidated, except for frequent TP53 mutations.

Aims: This study was designed to clarify the mutation profile of AEL distinct from the myelodysplastic/leukemia (MDS/AML) syndrome (MDS).

Methods: We performed a comprehensive genetic study, in which paired tumor/normal DNA from 22 AEL cases were analyzed using whole exome sequencing (WES). Whole-exome sequencing data from 3 AELs generated by The Cancer Genome Atlas (TCGA) was also included in the analysis. Subsequently, a total of 84 AEL cases were screened for mutations in 67 driver genes associated with myeloid malignancies using targeted-capture sequencing, in which RNA baits were also designed for a total of 1158 single nucleotide polymorphism sites to allow for genome wide copy number abnormalities and other allelic imbalances.

Results: Median age at diagnosis was 58.5 (21-87) years old. Among the 77 patients with clinical information available, 62 patients were diagnosed with de novo AML, 13 with secondary AML, and 2 with treatment-related AML. On average, 18.4 ± 3.4 mutations were detected per sample in whole-exome and targeted-capture sequencing in AEL, as compared to 12.2 ± 2.9 mutations (P = 0.014) in other AML, respectively. Both platforms being combined, most frequently observed was TP53 mutations (n=26, 31%) with complex karyotype being accompanied in most cases (25 cases), which were associated with a significantly shorter overall survival (P<0.001). Other frequent mutated genes were those encoding major components of the cohesin complex, including STAG2 (12%), SMC1A (4.8%) and RAD21 (2.4%), which were mutated in as high as 30% of the cases. The splicing machinery (18%) and epigenetic regulators (45%) were also important for MDS/AML, 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 epigenetic co-regulators, while STAG2 were mutually exclusive with double ENY, and IDH1/2. TP53 mutations were mutually exclusive with cohesin mutations (p=0.01) and those in epigenetic co-regulators, while STAG2 were mutually exclusive with double ENY, and IDH1/2.

Summary/Conclusions: TP53-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 MDS/AML
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Background: The Cohesin complex is an evolutionarily conserved multimeric protein complex, responsible for regulation of genome topology by facilitating the complex plays pivotal roles within mitosis and sister chromatid cohesion however, substantial data exists elucidating roles for the complex within the DNA damage response, homologous recombination and long-range interaction between cis regulatory elements of the genome. Within hematological malignancies, upwards of 20% of patients diagnosed with either Acute Myeloid Leukemia (AML), Myelodysplastic syndrome (MDS) or Myeloproliferative neoplasm (MPN) have been shown to harbour mutations within the Cohesin complex, with many more showing significantly reduced expression of core complex members.

Aims: To explore the impact of a loss of function STAG2 mutation on the chromatin architecture within a isogenic cell based model.

Methods: Using a CRISPR generated isogenic model we have investigated the impact loss of STAG2 has on the chromatin architecture of a hematopoietic environment. Genome wide binding profiles for STAG1, STAG2 and CTCF were generated using ChIP-Seq to elucidate areas of differential between STAG members. In addition, binding profiles for H3K27ac, H3K27me3 and H3K4me1 were generated using ChIP-Seq to provide genome wide identification of active and repressed enhancer regions, with the regions ranked to identify both normal and super-enhancer regions. These samples were matched to ATAC-Seq profiling of open and closed chromatin regions as well as RNA-seq samples to provide information on gene activity in relation to chromatin state in the absence of STAG2.

Results: Our results indicate that STAG1 binding profiles after following loss of function of STAG2, with an increase in binding peaks from ~17,000 to 25,000, however several sites identified by ChIP-Seq are not compensated for. Histogram mark profiling identified wide spread expansion of the H3K27ac mark and a decrease in regions of H3K27me3 consistent with loss of boundaries within topologically associated domains. This spread of an activator mark correlates with altered gene expression and the changes observed in ATAC-seq profiling of altered chromatin accessibility. The open chromatin regions identified through H3K27ac and H3K4me1 were lost in a compressive fashion and the cohesin complex was recruited in a competition factor occupancy. An enrichment for transcription factors relevant for myeloid transcriptional programmes was observed. This increase in enrichment aids in the suggestion that the impact of mutant/alternated cohesin complex function relates directly to the specific cell type and maturation state at which it occurs.

Summary/Conclusions: This research into the aberrant and non-canonical use of the Cohesin complex provides insights into the potential impact of mutant STAG2 on the spatio-temporal genomic architecture in hematological malignancies and begins to yield insight into the clinical implications of mutations within the cohesin complex.
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 MLL mutations are chromosome translocations that fuse the MLL gene in-frame with a second partner gene, creating novel fusion proteins (MLL-FPs). MLL-AF9 is the most common MLL-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 may be 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 H3K4me1 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 (3C) technique, Capture-C (one vs all) was used to further characterize interactions from the MYB promoter. We then performed siRNA knockdown of MYB and assessed the effect of MYB loss on its downstream druggable target BCL2, using RT qPCR, Western blotting and ChIP qPCR.

Results: We identified MLL-AF9 binding to novel putative enhancers of MYB as defined by regions co-bound by H3K27ac, H3K4me1 and marked by open chromatin on ATAC-seq. Furthermore, Capture-C from the MYB promoter identified novel putative enhancer-promoter interacting domains 100-200kb apart that are co-bound by MYB but not MLL-AF9. This suggests long-range autoregulation of MYB. Next, siRNA knockdown of MYB results in loss of MYB binding at the BCL2 promoter and its downstream enhancer by ChIP qPCR. There is a corresponding loss of BCL2 mRNA and protein expression in MYB knocked-down cells compared with control, confirming that BCL2 is directly regulated by MYB.

Summary/Conclusions: We have identified for the first time, regulation of MYB by MLL-AF9 via putative enhancers, and also an autoregulatory role of MYB involving long-range cis-interactions. Furthermore, we confirm that BCL2 is directly regulated by MYB in MLL-AF9 leukaemia, suggesting a molecular rational for using BCL2 inhibitors in MLL-AF9 leukaemia therapy.
We have recently discovered that FLT3-ITD+ AML cells are highly sensitive to the FDA-approved, cannabinoid (THC)-like compound WIN-55,212-2. This cannabinoid displays a selective and potent proapoptotic effect against leukemic cells. Several pathways are involved in this effect, the modification of the sphingolipids pattern playing a main role.

### P543

**CANNABINOIDS DERIVATIVES MODIFY THE PATTERN OF SPHINGOLIPIDS IN ACUTE MYELOID LEUKEMIA CELLS AND PRODUCE A POTENT ANTI-LEUKEMIC EFFECT**

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**Background:** Endocannabinoid system is a set of ligands, receptors and endogenous enzymes which modulate a variety of physiological effects. There are two well-characterized cannabinoid receptors, CB1 (mainly expressed in Central Nervous System) and CB2 (mainly in hematopoietic cells). Here, we tested the effect of the cannabinoid WIN-55 212-2 in acute myeloid leukemia (AML) in vitro and in vivo and studied the molecular signaling pathways involved in this effect, specially the role of sphingolipids. Moreover, we synthesized a new family of twelve cannabinoids that are specific to CB2 receptor.

**Aims:** - Development of new compounds derived from cannabinoids with CB2 selectivity and evaluation of their anti-tumor effect in AML in vitro and in vivo.

To deepen in the knowledge of lipid metabolism in AML.

**Methods:** For the design and synthesis of new cannabinoids, computational techniques of docking, analytical and spectroscopic techniques such as mass spectrometry (MS) were used. To assess the anti-leukemia effect of the different cannabinoids, we analyzed cell viability by MTT and flow cytometry using six biological replicates. To assess the anti-leukemia effect of cannabinoids, we analyzed cell viability by MTT and flow cytometry using six biological replicates. To assess the anti-leukemia effect of cannabinoids, we analyzed cell viability by MTT and flow cytometry using six biological replicates.

**Results:** Palbociclib impaired the viability of murine Baf3 cells with FLT3-ITD+<sup>1</sup> and inhibited the phosphorylation of the downstream proteins p-ERK 1/2, p-AKT, p-P38 and p-JNK. 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 not only due to cell cycle arrest as by transfection, concomitant with transcriptional activation of CDK6 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 kinase (AURK) and AKT. Thus, CDK6 inhibition upon AURK and AKT in mutant Baf3 cells, two signalling nodes critical for survival of tumor cells. Dual targeting with palbociclib and AURK or AKT inhibitors resulted in synergistic cytotoxicity.

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<image2>
with the Papaemmanuil dataset, we observed a weaker correlation for relapses after CT ($r^2=0.69$) and an even more marked deviation for post-transplant relapses ($r^2=0.45$). This difference was mainly explained by the enrichment in both relapse cohorts for FLT3-ITD (25% in diagnoses vs 55% and 48% at relapses after CT and allo-HSCT, $p < 0.01$ for both comparisons) and WT1 mutations (5% vs 25% and 22%, $p < 0.01$ for both comparisons). For 24 cases it was possible to longitudinally compare the mutational profile of AML at diagnosis and relapse in the same patient: we observed higher stability in relapses after CT, with 50% of cases carrying the same pattern of mutations present at diagnosis, whereas at relapses after allo-HSCT changes were more frequent, with 70% of patients displaying new gains or losses.

**Summary/Conclusions:** Taken together, our data evidence that the genomic landscape of AML at relapse can be significantly different from the one documented at diagnosis, suggesting that the selective pressure mediated not only by intensive chemotherapy, but also by the graft-versus-leukemia effect, can be potent drivers of clonal evolution. From the practical standpoint, the pattern of emergence of novel mutations that we documented should be taken into account not only for targeted salvage approaches, but also for the design of post-remission strategies aiming to prevent relapse.

P545

**Abstract withdrawn.**

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**Acute myeloid leukemia - Clinical 4**

**P546**

**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 associations between recurrent driver genetic mutations and other potential prognostic factors which might influence the clinical outcome.

Results: The median age in the total cohort was 76 years (y) (range: 75-86 y). 44% of pts reached complete remission (CR) and 4% CR with incomplete blood count recovery (CRi). The median overall survival (OS) was 6 months with a 3-year OS of 21% (by SWOG). Accordin,...

Background: We assessed GMI-1271 plus salvage chemotherapy with mitoxantrone, a Phase (Ph) 1 trial in pts with R/R AML escalated GMI-1271 across standard therapy. Patients are enrolled into 6 BST-236 escalating-dose cohorts (0.3-6 g/m²/day), each composed of 3-6 pts. Treatment was administered 1-hour daily infusion for 6 days. 

Methods: A Phase (Ph) 1 trial in pts with R/R AML escalated GMI-1271 across standard therapy. Patients are enrolled into 6 BST-236 escalating-dose cohorts (0.3-6 g/m²/day), each composed of 3-6 pts. Treatment was administered 1-hour daily infusion for 6 days. BST-236 is safe and very well tolerated, enabling delivery of high cytarabine doses to target cells with lower systemic exposure to the free drug and relative sparing of normal tissues. As such, BST-236 may serve as an ideal therapy for leukemia, particularly for delivering high doses of cytarabine to pts who are unfit or older. 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.

Results: To date, treatment of cohorts 1-5 is completed, with 18 patients treated from it. No neurological or grade >2 typical cytarabine events such as gastrointestinal toxicity, bone marrow suppression, and infections, leading to high treatment-related mortality rates. Hence, while the incidence of AML increases with age, advanced age and comorbidities may preclude the administration of intensive therapy altogether.

Aims: We assessed BMI, lymphocytosis, elevated E-sel and neutrophil to lymphocyte ratio as novel markers for chemotherapy response. In this study, we aimed to determine if BMI, lymphocytosis, elevated E-sel and neutrophil to lymphocyte ratio are novel markers for chemotherapy response.
P549
FEASIBILITY AND BENEFIT OF TARGETED RNA SEQUENCING FOR THE DETECTION OF RECURRENT FUSION TRANSCRIPTS AND THE IDENTIFICATION OF NOVEL FUSION TRANSCRIPTS IN MYELOID MALIGNANCIES

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Background: Fusion genes are frequent genetic abnormalities in myeloid malignancies. The impact of the detection of such gene fusions is rising due to an increasing number of drugs targeting them as has been impressively shown for e.g. BCR-ABL1 and PML-RARA. Further, they can be used as biomarkers for disease monitoring.

Aims: Evaluation of targeted RNA sequencing for the detection of recurrent and novel fusion transcripts.

Methods: 102 cases with myeloid malignancies harboring 105 translocations identified by chromosome banding analysis were selected. Recurrent fusion genes had been confirmed by FISH and/or RT-PCR. In cases with suspected novel fusions the rearrangement of one partner gene had been confirmed by FISH. The following recurrent rearrangements identified by standard diagnostic procedures were present: PML-RARA (n=11), RUNX1-RUNX1T1 (n=7), CSF3R-MYH11 (n=3), KMT2A-ELL (n=4), KMT2A-MLLT10 (n=3), KMT2A-MLLT3 (n=3), KMT2A-MLLT4 (n=2), BCR-ABL1 (n=3), NUP98-NSD1 (n=3), DDX3-NUP214 (n=1), and KAT6A-CREBBP (n=1). Further, cases harboring MMSET-KMT2A (14), RUNX1 (n=1), ET1V (n=6), PDGFRB (n=10), RARA (n=2), NPM1 (n=2), and NUP98 (n=1) were included. Targeted RNA sequencing was performed using the TruSight RNA Fusion panel (Illunina, San Diego, CA) consisting of 7690 probes covering 507 genes known to be involved in gene fusions. Library was prepared according to manufacturer’s protocol with ~50ng RNA extracted from fresh/frozen samples. Sequencing was performed on the NextSeq instrument (Illunina) and analysis with the RNA-Seq Alignment App (BaseSpace Sequence Hub) using Star for Alignment and Manta for gene fusion calling with default parameters (illumina).

Results: In 42/45 (93%) cases with a recurrent rearrangement identified by standard diagnostics, RNA sequencing detected the respective fusion transcript. In addition, RNA sequencing was able to identify known and novel fusions in the remaining 57 cases. For KMT2A these were the following partner genes: MLLT1 (n=5), ELL (n=3), ITF2, FLNC, ASXL2, DCP1B, MAML1 and ARHGEF12. Seven different partner genes were identified in RUNX1 translocations: PLAG1 (n=2), PRDM16, MECOM, ZFPM2, MAN1A2, NAM2T, and KIAA1549L. Five different partner genes were identified in ETV6 rearranged cases: ABL1, CCDC126, ERG, FOXO1 and CFLAR-AS1. Most strikingly was the identification of the ETV6-ABL1 fusion, which could not be suspected by cytopathology as the 5 ETV6 FISH signal was located on chromosome 17. In 7107D-PDGFRB rearranged cases the partner genes were identified. These were WDR4, CCDC88C, MPRIP, TNIP1, TFR, NF1 and ZBTB11. Further the following fusions were found: NPM1-RPPO, NPM1-SETBP1, NUP98-ING3, IRF2BP1-PARA, and ZBTB16-RARA. Thus, RNA sequencing identified 39 fusions which standard diagnostics had failed to detect, of which one-third of the partner genes were not identified in the respective cases. Failure to detect gene fusions should initiate improvements in calling algorithms and may also have biological reasons. It was reported that genomic rearrangements of one of the partner genes. Failure to detect gene fusions should initiate improvements in calling algorithms and may also have biological reasons. It was reported that genomic rearrangements of RUNX1 occur, which do not lead to RUNX1 in frame fusion transcripts but to truncation of transcription.

Summary/Conclusions: 1) RNA sequencing was able to detect recurrent gene fusions with high accuracy and to characterize rare gene fusions providing the basis for the design of RT-PCR based assays for monitoring MRD. 2) Targeted RNA sequencing may be a valuable tool in routine diagnostics for patients with rearrangements unresolved by standard techniques. 3) These findings may have consequences for targeted treatment approaches.

P550
COMPREHENSIVE MOLECULAR ANALYSIS OF ADULT MIXED PHENOTYPE ACUTE LEUKEMIA (MPAL)

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3The University of Texas MD Anderson Cancer Center, Texas, United States
4Department of Hematology, The University of Texas MD Anderson Cancer Center, Texas, United States

Background: Mixed phenotype acute leukemia (MPAL) is a rare subgroup of acute leukemia characterized by blasts that show immunophenotypes of both myeloid and lymphoid lineages and therefore not traceable to single lineage of origin. Diagnosis of MPAL is challenging due to the possible discrepancy between immunophenotype and morphology. Clinically, MPAL has poor prognosis and poses therapeutic challenges. Genetic basis of MPAL is not well understood.

Aims: To clarify the underlying pathogenesis of MPAL and provide clue on future personalized therapy in MPAL, we performed comprehensive molecular characterization of adult MPAL.

Methods: We studied 31 patients with adult MPAL (median age 53 years) that met 2008 WHO criteria for lymphoblastic leukemia/lymphoma. Targeted RNA sequencing was performed using the TruSight RNA Fusion panel. Forty-four cases were analyzed by targeted capture exome sequencing of 295 genes that are recurrently mutated in hematologic malignancies (median 393x coverage, N=31), RNA sequencing (N=24), and NGS methylated genomic DNA (median 39x coverage, N=31). Mutational landscape was compared to that of 194 AML, 71 B-ALL, and 6 T-ALL. Cases in which pre-treatment samples were available were analyzed by targeted capture and whole exome sequencing (n=11). RNA sequence analysis was performed using 2000 Exome Plus Assay (Illumina, San Diego, CA) consisting of 7690 probes covering 507 genes known to be involved in gene fusions.

Results: Targeted RNA sequencing revealed the presence of one or more focal recurrent gene fusions in 20 (65%) cases, including KMT2A (n=6), NUP98 (n=4), KMT2A (n=3), MYC (n=2), and NUP214 (n=2). Mutational analysis of myeloid sample revealed that genomic rearrangements of one of the partner genes. Failure to detect gene fusions should initiate improvements in calling algorithms and may also have biological reasons. It was reported that genomic rearrangements of RUNX1 occur, which do not lead to RUNX1 in frame fusion transcripts but to truncation of transcription.

Summary/Conclusions: 1) RNA sequencing was able to detect recurrent gene fusions with high accuracy and to characterize rare gene fusions providing the basis for the design of RT-PCR based assays for monitoring MRD. 2) Targeted RNA sequencing may be a valuable tool in routine diagnostics for patients with rearrangements unresolved by standard techniques. 3) These findings may have consequences for targeted treatment approaches.

P551
THE EFFECTS OF EARLY INTENSIFIED INDUCTION CHEMOTHERAPY IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA COMPARED TO STANDARD ANTHRACYCLINE PLUS CYTARABINE 3+7 CHEMOTHERAPY D.-H. Kwak1, H.-J. Yoon1, H.-J. Kim1, S.-S. Park1, S.-E. Lee1, B.-S. Cho1, K.-S. Eom1, Y.-J. Kim1, S. Lee1, J.-W. Lee1, W.-S. Min1

1Department of Hematology, Catholic Blood and Marrow Transplantation Center, Leukemia Research Institute, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic Of

Background: Standard induction chemotherapy for acute myeloid leukemia (AML) which consists of anthracycline for 3 days plus cytarabine for 7 days was first introduced in 1970’s and has been used for a long time. Several modification or intensification for this conventional regimen did not prove the effect for higher complete remission (CR) rate or lower relapse rate which led to superior overall survival (OS) rate.

Aims: We tried to find out possible benefit of early intensification of standard induction chemotherapy in adult AML patients.

Methods: The present study enrolled 1195 adult AML patients from 2002 to 2013. All patients were initially treated with idarubicin (12mg/m2) plus cytarabine (100mg/m2) plus daunorubicin (25mg/m2) induction chemotherapy (3+7), and among them, 731 (61.2%) patients received additional early augmentation using cytarabine 3 days (3+10, n=363) or anthracycline 2 days plus cytarabine 3 days (2+11, n=348). Outcome of 2 groups was compared, which was based on the follow-up BM blast counts on the 7th day of 3+7 chemotherapy. Totally 3+10 for blast counts 5%–20% and 5% for blast counts >20% (early intensified group). The rest 464 with blast counts < 5% finished with 3+7 regi-
men (standard group). Re-induction and consolidation therapy was performed according to a consistent strategy and post-consolidation 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 73 [15.7%] vs 3+10 [12.9%], P<0.001). Also, initial GM blast counts were higher in two intensified groups (73.3% in 3+10 and 70.1% in 3+10 compared to 3+7 subgroup. (66.8%, P<0.001). Early death rate at 8 weeks was higher in patients older than 55 years (10.8% vs 3.7%, P<0.001) especially when they were treated with intensified chemotheraphy (21.7% in 3+10 and 15.7% in 3+10 vs 6.3% in 3+7, P=0.038). CR rate after induction was higher in young patients especially in 3+10 subgroup (79.8%, P<0.001) and we also found that patients with favorable to intermediate-risk karyotype might benefit with intensified chemotherapy in the context of CR rate (79.7% vs 68.3%, P<0.001, although final CR rates became similar after re-induction. Next, we found that pre-CHT relapse was lower in patients younger than 55 years (4% vs 0%, P=0.002) and favorable to intermediate-risk group (8.9% vs 20.2%, P<0.001) after intensified induction. In young patients with favorable to intermediate-risk karyotype, intensified groups showed superior 5-year OS (55.0% vs 45.5%, P=0.010) and lower long-term relapse rate (32.2% vs 38.0%, P=0.094), but multivariate analysis revealed no effects for both OS and CR. In patients older than 55 years, intensified groups showed inferior 5-year OS (19.2% vs 22.8%, P=0.014) with higher early death rate (17.6% vs 6.3%, P=0.015), and multivariate analysis also showed intensified induction was related inferior OS (HR=1.89, 95%CI; 1.14-3.15, P=0.013).

### Table 1

<table>
<thead>
<tr>
<th>Patient Age</th>
<th>OS Rate</th>
<th>CR Rate</th>
<th>CR after induction (%)</th>
<th>Early death rate (%)</th>
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<tr>
<td>&lt;50 years</td>
<td>70/112</td>
<td>28/67</td>
<td>22/77 (27.3)</td>
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<td>0.001</td>
</tr>
</tbody>
</table>

### Summary/Conclusions:

Our data revealed that intensified induction chemotherapy was not influential for poor-risk karyotype, while higher post-induction CR rate and low pre-CRT 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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1Department of Medicine, Roswell Park Cancer Institute, Buffalo, 2Department of Medical Oncology, Dana-Farber Cancer Institution in additional 6 pts. Out of 29 newly diagnosed FLT3 mutant AML patients with full/partial FLT3 gene sequencing performed, 4 pts were found to have novel variant FLT3 mutations consisting of V491L, V592L, D593H, A680V, and N841IT/K (Table 1). The majority of these novel mutations were located at the JM, kinase domain 1 and the activation loop (kinase domain 2). The allele fractions of these FLT3 variants ranged as high as 29% (higher than that of FLT3-ITD in pt3), suggesting that some of these clones may have been potentially driving clinical leukemia progression in some pts. All 4 pts had NPM1 mutations, and two also had DNMT3A mutations. All 4 pts achieved CR with full count recovery (3/4 pts achieved CR after just one cycle of cytarabine/anthracycline/crenolanib induction). The pt with FLT3-D835Yand N841IT achieved CR after cytarabine/anthracycline/crenolanib induction and one cycle of HiDAC consolidation. All pts received FLT3-ve and have remained CR-3-ve out of 4 pts received 1-4 cycles of HiDAC consolidation follow 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.

### Table 1

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<td>70/70</td>
<td>70/70</td>
<td>70/70</td>
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</table>

### Summary/Conclusions:

This abstract reports multiple novel variant FLT3 mutations in adult pts with newly diagnosed FLT3-ITD or FLT3-D835 mutant AML. The allelic burden of these FLT3 variant mutations can sometime be higher than that of FLT3-ITD. Detailed FLT3 analyses in this subset of pts suggests that 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 ITD, 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 IDH mutation in AML have generally reported inferior survival compared to other patients with AML. More recently, a small single institution study suggested that patients with IDH mutation in AML could benefit from induction chemotherapy with the 7+3 regimen. However, this study did not consider the prevalence of complex karyotype and FLT3-ITD in patients with IDH mutation.

**Objective:** To evaluate the outcomes of newly diagnosed AML with IDH1 or IDH2 mutations who have undergone induction chemotherapy with “7+3” regimen.

**Methods:** We retrospectively reviewed the records of 115 patients with AML from the Memorial Sloan Kettering Cancer Center who initiated induction chemotherapy between 2001 and 2014. Patients with IDH1 or IDH2 mutations were identified. The primary endpoint was OS. Pre-treatment characteristics and outcomes were compared between patients with IDH mutation without complex karyotype or FLT3-ITD (‘‘IdH1/2+’’), patients with complex karyotype (‘‘K+’’), and patients with FLT3-ITD (‘‘ITD’’).

**Results:** A total of 26 patients with IDH1/2 mutations were identified. The median age of patients was 59 years (range 32-79). The majority of patients were male (62%) and had a high intergroup risk (69%). The most common type of IDH mutation was IDH2-R172K (77%). The most common cytogenetic abnormalities were +8 (23%) and t(8;21) (19%). The majority of patients (65%) received induction chemotherapy with “7+3.” The OS of patients with ‘‘IdH1/2+’’ was significantly better than patients with ‘‘K+’’ (P=0.019) and patients with ‘‘ITD’’ (P=0.03). The OS of patients with ‘‘IdH1/2+’’ was not significantly different from patients with ‘‘IdH1/2-’’ (P=0.16).

**Conclusion:** IDH mutation is an independent predictor of OS in newly diagnosed AML patients who have undergone induction chemotherapy with “7+3”. Patients with IDH mutation who are eligible for induction chemotherapy with “7+3” have a better OS compared to patients with complex karyotype and FLT3-ITD. This study supports the use of “7+3” in newly diagnosed patients with IDH mutation in AML.

**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 ITD, D835, as well as other activating mutations maybe beneficial.
Aims: To delineate the complete remission rate in AML patients with IDH1 or IDH2 mutations who receive standard 7+3 induction chemotherapy.

Methods: After receipt of IRB approval, an institutional database of genomic abnormalities in all patients with AML was queried for patients with IDH1 or IDH2 mutations between the years of 2010 and 2016. Pathology records of patients identified as having an IDH1/IDH2 mutation were reviewed to confirm the presence of an IDH mutation. After confirmation of IDH mutational status, all patients who received standard induction chemotherapy with 7+3 were included in this retrospective chart review.

Results: Between 2010 and 2016, 82 patients with IDH1/IDH2 mutations who had been treated with 7+3 induction chemotherapy were seen at MSKCC. Of these, 33 (40.2%) had IDH1 mutations and 49 (59.8%) had IDH2 mutations. Of those with IDH2 mutations IDH2 R140Q mutations were present in 34 (69.3%), and IDH2 R172K mutations were present in 15 (30.6%). The median age of all patients treated was 63. 56 patients (68%) had de novo AML, 16 (20%) had AML with myelodysplasia related changes, 5 (6%) had a known prior history of MDS and 5 (6%) had therapy related AML. Nearly half of the patients (49%) had karyotypic abnormalities. Of the 82 patients who received induction chemotherapy with 7+3, 51 achieved a complete remission (CR) after 1 cycle and 16 after 2 cycles for a CR rate of 82%. The strongest predictor of response to induction chemotherapy was the presence of an NPM1 mutation. There was a trend towards decreased response to induction chemotherapy in patients with a complex karyotype (p=0.079) that did not reach statistical significance. The presence of an IDH2 R172K mutation was predictive of non-response to one cycle of (7+3) of 7+3 but when two cycles of induction chemotherapy were given, response rates were equivalent to patients with R140Q mutations. Co-occurring mutations in FLT3 (ITD or TKD), DNMT3A or N-RAS 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 IDH1 or IDH2 mutation. CR is not affected by FLT3 ITD or TKD, although those 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 NRAS mutations. For AML patients with IDH mutations who are eligible for induction chemotherapy, 7+3 is a reasonable induction regimen regardless of the presence of FLT3 mutations, or karyotypic abnormalities.
However, the correlation is only valid for CYT-IDA while the PM Test is applied to up to 45 treatments. Any such treatment identified as sensitive means the PM Test can provide a valuable guideline to hematologists. This means the PM Test can suggest sensitive treatments for the vast majority of patients.

**Figure 1.**

Summary/Conclusions: We have developed a novel ex vivo PM test for induction treatment in AML patients to guide hematologists selecting the right treatment to achieve CR in individual patients leveraging up to 45 different validated chemotherapeutic regimes. Assuming a similar response rate for all these treatments, our test could estimate a net prediction for sensibility to AML treatment higher than 80% in 1st line. This PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

**P555**

RESPONSE-ADAPTED AZACITIDINE AND INDUCTION CHEMOTHERAPY IN PATIENTS >60 YEARS OLD WITH NEWLY DIAGNOSED AML ELIGIBLE FOR CHEMOTHERAPY: RESULTS OF THE DRKS00004519 STUDY OF THE EAST GERMAN STUDY GROUP

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1Department of Hematology and Oncology, University Hospital of Leipzig, 2Department of Hematology and Oncology, University of Leipzig, Leipzig, 3Department of Hematology and Oncology, Klinikum Chemnitz, Chemnitz, 4Department of Hematology, Oncology and Palliative Care, Klinikum Ernst von Bergmann, Potsdam, 5Department of Hematology, Oncology and Palliative Care, Klinikum Magdeburg, Magdeburg, 6Department of Hematology and Oncology, University Hospital of Halle, Halle (Saale), 7Department of Hematology and Oncology, Helios Klinikum Erfurt, Erfurt, 8Clinical Trial Centre Leipzig, University of Leipzig, Leipzig, Germany

Background: AML treatment in elderly patients (pts) >60 years (y) with intensive chemotherapy (IC) or azacitidine (AZA) are not necessarily mutually exclusive. Aims: Results of the multicenter DRKS00004519 (RAS-AZIC) study of the East German Study Group (OSHO) which evaluated first-line treatment with AZA followed by response-based AZA or IC in pts >60y with AML are presented. Methods: pts >60y with newly diagnosed AML (n=112) were included. Recruitment was completed in May, 2016. In the phase 1 part, safety of upfront AZA (75mg/m²/day s.c) for 7 days followed by IC (mitoxantrone 10mg/m²/day on day (d) 1-3 and cytarabine 1g/m²/BID on d 1, 3, 5, 7) on d17 was established through a 3+3 design. In the multicenter phase II part (figure), upfront AZA was sequentially followed by AZA or IC based on d15 bone marrow (BM) blasts (<45 vs ≥45%) and CR/CRi on d56 which were both previously identified as early predictors for long-term response to AZA in AML (Ai-All et al. Leuk Lymph 2011). The primary endpoint was response (CR/CRi, and PR) and OS according to the International Working Group criteria. Based on the optimal two-stage design (Simon. Control Clin Trials 1989), protocol treatment was non-inferior to standard IC if, on an intention-to-treat basis, an OR of 61% was reached. Adverse events (AEs) were reported according to the NCI CTCAE 4.03. All pts gave written informed consent. Results: Median age was 70y (52% males), de novo AML was present in 65% of pts. Median BM blasts and WBC were 50% and 4.4x10⁹/L respectively. Genetic risk was high in 30%, intermediate in 55%, and favorable in 15%. FLT3 and NPM1 were mutated in 12% and 22% respectively. All pts received first-line AZA. Only lower baseline blasts correlated with 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 AML cycles given till d56, 33.6% were applied in an outpatient setting. Until d90, one IC cycle was needed in 77 (68.8%) pts. In the intention-to-treat cohort (n=43%/15%); PR (4.5%)

**Figure 1.**

Summary/Conclusions: We have developed a novel ex vivo PM test for induction treatment in AML patients to guide hematologists selecting the right treatment to achieve CR in individual patients leveraging up to 45 different validated chemotherapeutic regimes. Assuming a similar response rate for all these treatments, our test could estimate a net prediction for sensibility to AML treatment higher than 80% in 1st line. This PM Test will be evaluated in an interventional clinical trial on relapse/refractory patients that is expected to begin in the next few months in collaboration with the PETHEMA group from Spain.

**P555**

OVERALL SURVIVAL WITH CPX-351 VERSUS 7+3 IN OLDER ADULTS WITH NEWLY DIAGNOSED, THERAPY-RELATED ACUTE MYELOID LEUKEMIA: SUBGROUP ANALYSIS OF A PHASE 3 STUDY

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Background: Therapy-related acute myeloid leukemia (tAML) may occur as
a late complication of cytotoxic or radiation therapy and is associated with a poor prognosis. CPX-351 is a liposomal formulation that delivers a synergistic 5:1 molar ratio of cytarabine and daunorubicin. In a randomized, open-label, controlled phase 3 trial in patients aged 60 to 75 years with newly diagnosed, secondary AML (eg, tAML or AML after myelodysplastic syndrome), CPX-351 significantly improved overall survival (OS) versus cytarabine/daunorubicin (7+3).

**Aims:** The current analysis of this phase 3 study evaluated outcomes in the subgroup of patients with tAML.

**Methods:** Enrolled patients were randomized 1:1 to receive induction with 1 to 2 cycles of CPX-351 (100 units/m² [cytarabine 100mg/m² + daunorubicin 44mg/m²] on Days 1, 3, and 5 [2nd induction: Days 1 and 3 only]) or 7+3 (cytarabine 100mg/m²/day x 7 days [2nd induction: x 5 days] + daunorubicin 60mg/m² on Days 1, 2, and 3 [2nd induction: Days 1 and 2 only]). Patients who achieved complete remission (CR) or CR with incomplete platelet or neutrophil recovery (CRi) could receive up to 2 cycles of consolidation therapy. Note, the study was not powered for this subgroup analysis.

**Results:** A total of 304 patients were enrolled and received study treatment, including 62 (20%) patients with tAML (CPX-351 arm, n=30; 7+3 arm, n=32). Characteristics of tAML patients were similar between the CPX-351 and 7+3 arms: median age was 69.0 versus 67.5 years, and 47% versus 53% were male. Primary 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 36%, respectively; odds ratio=1.33 [95% CI: 0.47, 3.81]) and proceeded to stem cell transplantation (37% vs 27%; odds ratio=1.54 [95% CI: 0.53, 4.49]). Serious treatment-emergent adverse events (TEAEs) were reported for 18/30 (60%) of tAML patients in the CPX-351 arm and 12/32 (38%) of tAML patients in the 7+3 arm; the observed difference in serious TEAEs in this subgroup appeared to primarily be due to the incidence of febrile neutropenia (n=6/30 [20%] vs n=0/32 [0%]).

Three (10%) patients in the CPX-351 arm and 5 (16%) patients in the 7+3 arm experienced a TEAE that resulted in death during the treatment period; there was no pattern in the individual TEAEs that led to death.

**Figure 1.**

**Summary/Conclusions:** CPX-351 is associated with improved efficacy and a safety profile comparable to 7+3 in older patients with newly diagnosed tAML. Outcomes in the tAML subgroup mirrored the overall study population, indicating CPX-351 may represent a new therapeutic option for this difficult to treat population.

**P557**

HYPERFERRITINEMIA IS AN INDEPENDENT POOR PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA

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**Background:** The prognostic impact of ferritinemia has been studied in a variety of malignancies, including acute myeloid leukemia. Ferritin is a protein that is upregulated in response to tissue iron stores and inflammatory conditions. In a previous study, ferritin was found to be associated with an increased risk of relapse and death in patients with acute myeloid leukemia (AML).

**Methods:** In the current analysis of this phase 3 study evaluated outcomes in the tAML subgroup. Outcomes in the tAML subgroup mirrored the overall study population, indicating an improved efficacy and a safety profile comparable to 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 36%, respectively; odds ratio=1.33 [95% CI: 0.47, 3.81]) and proceeded to stem cell transplantation (37% vs 27%; odds ratio=1.54 [95% CI: 0.53, 4.49]).

Serious treatment-emergent adverse events (TEAEs) were reported for 18/30 (60%) of tAML patients in the CPX-351 arm and 12/32 (38%) of tAML patients in the 7+3 arm; the observed difference in serious TEAEs in this subgroup appeared to primarily be due to the incidence of febrile neutropenia (n=6/30 [20%] vs n=0/32 [0%]). Three (10%) patients in the CPX-351 arm and 5 (16%) patients in the 7+3 arm experienced a TEAE that resulted in death during the treatment period; there was no pattern in the individual TEAEs that led to death.

**Figure 1.**

**Summary/Conclusions:** In conclusion, hyperferritinemia is a prognostic marker independent from well-acknowledged factors, such as cytogenetics and molecular abnormalities. Ferritinemia should be included in AML diagnosis workup as it provides reproducible information on short and long-term outcome for AML patients of any subgroup. The putative link between hyperferritinemia, inflammation and chemoresistance should be investigated.

**P558**

NGS ANALYSIS OF 474 BONE MARROW SAMPLES FROM 157 AML PATIENTS TREATED WITH AZACITIDINE–IMPACT OF AGE ON MUTATIONAL LOAD

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**Background:** Next-generation sequencing (NGS) is a powerful tool for detecting mutations in cancer, including acute myeloid leukemia (AML). The impact of age on mutational load in AML patients treated with azacitidine is not well understood.

**Methods:** A total of 474 bone marrow samples from 157 AML patients treated with azacitidine were analyzed using NGS. The impact of age on mutational load was assessed using linear regression models.

**Results:** Age was found to have a significant impact on mutational load, with younger patients having a higher mutational load compared to older patients. The impact of age on mutational load persisted after adjusting for other factors, such as cytogenetic risk and prior treatment.

**Conclusion:** Age is an important factor in determining mutational load in AML patients treated with azacitidine. This finding may have implications for treatment strategies and patient selection.

**References:**

AML pts treated with azacitidine (AZA) has only been presented in abstract form thus far (Tang et al., ASH 2016). Data on the impact of age on mutational load in AML are scarce.

Aims: To assess the mutational landscape in elderly AML pts treated with AZA; specifically, whether age has an impact on mutational load.

Methods: We analysed 474 bone marrow FFPE specimens from 157 AML pts in the Austrian Registry of Hypomethylating Agents from two centres (Salzburg, Wels-Grieskirchen) using a 53-gene panel (all exons). NGS was performed by Qiagen. Minimum coverage: 1.500x. All mutations were checked against COSMIC-v79, ClinVar, ICGC, DoCM, dbSNP and Varsome databases. For comparison of categorical variables Chi-squared test was used, for comparison of means Student T-test was used.

Results: The rate of secondary (s)AML was significantly lower in pts <75 (n=85), vs ≥75 years (n=54) (60.6% vs 77.8%, P<0.001). There was no significant difference in the rate of adverse cytogenetics or monosomal karyotype before AZA treatment between pts < vs ≥75 years, respectively (data not shown). Mutational load (average number of mutated genes and mutations per pt) assessed at/before initiation of AZA, was significantly higher in pts <75 yrs vs ≥75 yrs (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 time point during the course of AML (including during/post-AZA treatment) (Figure 1B). In total, 139 pts had more than one marrow sample with NGS results. Analysis of paired samples revealed that mutational load was significantly higher during/post-AZA vs before AZA in both age groups (Figure 1C-D). In total, 60.4%, 15.8%, 8.6%, 3.6% and 11.5% of pts acquired 1, 2, 3, 4-13 additional mutations, respectively. No relevant differences between pts < vs ≥75 years were found (data not shown). When comparing the deltat of mutations before vs during/after AZA according to age group, no significant difference was found (Figure 1E).

Table 1.

| P559 PROGNOSTIC VALUE OF EARLY WT 1 RESPONSE IN AML PATIENTS UNDERGOING INTENSIVE CHEMOTHERAPY |

S. Mach camerl-Spandl1, 2, T. Vockenhuber1, M. Binder2, O. Zach1, M. Girschikofsky1, P. Beltzer1, A. Welter2,9,20,18,19,17,16,15,14,13,12,11,10,9,8,7,6,5,4,3,2,1, J. Altman2, A. Perl3, J. Cortes4, J. Hill5, R. Larson6, C. Liu7, E. Ritchie7, S. Strickland8, E. Wang1, A. Neubauer2, G. Martinielli1, E. Bahceci2

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Background: Monitoring minimal residual disease based on quantitative PCR represents an important risk stratification tool in acute myeloid leukemia (AML) and enables the prediction of impending relapse. Besides common fusion genes and mutated genes, Wilms tumor 1 (WT1) gene is widely used to follow de novo AML.

Aims: The aim of our study was to evaluate the relevance of WT1 expression for the prognosis of patients with AML in a real life population.

Methods: Bone marrow samples from 174 consecutive adult AML patients (18-85 years) were used for WT1 mRNA quantification. APL patients were excluded. Of 143 patients with WT1 overexpression at diagnosis, those treated with intensive induction chemotherapy and achieving haematological remission after the first cycle of therapy were included in the retrospective follow-up analysis (n=129).

Results: The extent of WT1 expression at diagnosis had no prognostic relevance. In contrast, achievement of low WT1 levels after induction chemotherapy was associated with a significant better overall (OS) and disease free survival (DFS) as compared to persistent high WT1 expression at hCR1 5 years OS 80% (95% CI 75.0-82.0) vs 69% (95% CI 60.7-73.9), DFS 90% (95% CI 86.7-91.0) vs 80% (95% CI 75.0-82.0), respectively (p<0.001). Additionally, compared with patients with a low WT1-reduction (<5 log) at hCR1, the relative risk of death was 0.32 (95% CI 0.1-0.7) in patients with intermediate WT1-reduction (5-8 log) and 0.15 (95% CI 0.00-0.5) in patients with high WT1-reduction (>8 log), after adjustment for age, ELN-risk group, and stem cell transplantation in CR1. The corresponding 5-year OS rates were 77.8% vs 84% (p=0.001) and 71% vs 89% (p<0.001), respectively. Even though numbers of patients were small (n=33), SCT at CR1 seems to overcome the adverse risk of persistent WT1 expression: DFS 5.3 years (0-12.9) for patients with SCT and 0.7 years (0-6.9) for patients without SCT (p=0.004).

Summary/Conclusions: Persisting WT1 expression in AML patients achieving a CR1 after induction chemotherapy is a strong, independent predictor for DFS and OS in patients with AML. Since 80–90% of AML patients exhibit WT1 overexpression at diagnosis, this marker is widely applicable for early risk re-evaluation and corresponding therapy adaptation.

P560 EVALUATION OF THE IMPACT OF SIGNAL RATIO ON OVERALL SURVIVAL IN FLT3-MUTATION-POSITIVE RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA FOLLOWING ONCE-DAILY TREATMENT WITH GILTERITINIB


1John Hopkins University, Baltimore, 2Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, 3University of Pennsylvania-Abramson Comprehensive Cancer Center, Philadelphia, PA, 4University of Texas Southwestern Medical Center, Dallas, TX, 5University of California-San Francisco, San Francisco, CA, 6Mayo Clinic, Rochester, MN, 7Astellas Pharma US Inc., Northbrook, IL, 8University of Chicago, Chicago, 9Weill Cornell Medical College, New York, NY, 10Vanderbilt-Ingram Cancer Center, Nashville, TN, 11Roswell Park Cancer Institute, Buffalo, NY, United States, 12Universitaetsklinik Giessen und Marburg, Marburg, Germany, 13Saragoni Institute of Hematology, Bologna, Italy

Background: Fms-like tyrosine kinase 3 (FLT3) internal tandem duplications (ITD) in acute myeloid leukemia (AML) are associated with early relapse and short survival, particularly in the context of high allelic burden. Patients with high FLT3-ITD signal ratio are particularly sensitive to FLT3 inhibitors but the clinical effects of allelic burden on survival have not been validated in trials of these drugs. Gilteritinib is a highly specific, potent FLT3/AXL inhibitor with demonstrated activity against both FLT3-ITD and tyrosine kinase domain (TKD) mutations. A recent Phase 1/2 study (CHRYSALIS; NCT02014558) demonstrated that FLT3 mutation-positive (FLT3mut+) patients with relapsed/refractory (R/R) AML treated with gilteritinib had high clinical response rates and prolonged overall survival (OS), especially at doses ≥80mg/d.

Aims: To evaluate the effect of FLT3-ITD and FLT3-TKD signal ratios on OS in AML patients who had received gilteritinib doses ≥80mg/d.

Methods: Signal ratios were assessed in adult FLT3mut+R/R AML pts who had received gilteritinib doses ≥80mg/d. Genomic DNA extraction and PCR with fluorescent primers were used to generate transcripts of FLT3 alleles containing ITD and TKD mutations. FLT3 alleles containing ITD mutations generating PCR products >330 bp in length were used to amplify the TKD region were used to amplify the target region and an EcoRV endonuclease was used to digest the PCR products >330 bp in length. Multiplex PCR products >330 bp in length were used in the PCR reactions and an EcoRV endonuclease was used to digest the PCR products >330 bp in length. Sequencing (The Cancer Genome Atlas Research Network, NEJM 2013; Merkelscheid1, A. Welter2,9,20,18,17,16,15,14,13,12,11,10,9,8,7,6,5,4,3,2,1, J. Altman2, A. Perl3, J. Cortes4, J. Hill5, R. Larson8, C. Liu9, E. Ritchie7, S. Strickland10, E. Wang11, A. Neubauer12, G. Martinielli13, E. Bahceci2

1John Hopkins University, Baltimore, 2Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Chicago, 3University of Pennsylvania-Abramson Comprehensive Cancer Center, Philadelphia, PA, 4University of Texas Southwestern Medical Center, Dallas, TX, 5University of California-San Francisco, San Francisco, CA, 6Mayo Clinic, Rochester, MN, 7Astellas Pharma US Inc., Northbrook, IL, 8University of Chicago, Chicago, 9Weill Cornell Medical College, New York, NY, 10Vanderbilt-Ingram Cancer Center, Nashville, TN, 11Roswell Park Cancer Institute, Buffalo, NY, United States, 12Universitätsklinik Giessen und Marburg, Marburg, Germany, 13Saragoni Institute of Hematology, Bologna, Italy

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Aims: To evaluate the effect of FLT3-ITD and FLT3-TKD signal ratios on OS in AML patients who had received gilteritinib doses ≥80mg/d.
P561

CLINICAL OUTCOME OF HYPOCELLULAR AML AND AML WITH MEYELODYSPLASIA-RELATED CHANGE (MRC) COMPARED TO DE NOVO ADULT AML WITH NORMAL CELLULARITY AFTER HEMATOPOIETIC CELL TRANSPLANTATION

D.-H. Kwak1, J.-H. Yoon1, H.-J. Kim1, S.-S. Park1, S.-E. Lee1, B.-S. Cho1, K.-S. Eom1, Y.-J. Kim1, S. Lee1, J.-W. Lee1, W.-S. Min1

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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) and AML-MRC was identified with at least two 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: 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 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 1.

Figure 2.

Summary/Conclusions: The long-term outcome of hypo-aML and AML-MRC were poorer than normocellular de novo AML, mainly due to older age and large proportion of adverse-risk karyotype which caused unavailable condition for HCT.

P562

INITIAL RESULTS FROM A FIRST-IN-HUMAN STUDY OF IMGN779, A CD33-TARGETING ANTIBODY-DRUG CONJUGATE (ADC) WITH NOVEL DNA ALKYLATING ACTIVITY, IN PATIENTS WITH RELAPSED OR REFRACTORY AML

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1MD Anderson Cancer Center, Houston, 2Dana-Farber Cancer Institute, Boston, 3Roswell Park Cancer Institute, Buffalo, 4University of New Mexico Cancer Center, Albuquerque, 5ImmunoGen, Inc., Waltham, 6University of Alabama at Birmingham, Birmingham, 7The Ohio State University, Columbus, 8Oregon Health and Science University, Portland, United States

Background: Acute myeloid leukemia (AML) accounts for the highest number of leukemia deaths in the United States annually. IMGN779 is an ADC that binds with high affinity and specificity to CD33, a validated therapeutic target in AML. IMGN779 comprises a humanized anti-CD33 antibody attached via a cleavable linker to the novel DNA-interacting payload DGN462. Once released when administered to patients with CD33+AML, Evaluation of the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary antitumor activity of IMGN779 are secondary objectives.

Methods: Adult patients (≥18 years) with relapsed or refractory CD33+ AML (defined by ≥20% of AML blasts expressing CD33 by flow cytometry) were eligible for enrollment. Informed consent was obtained from all patients. Dose-escalation, which follows a standard 3+3 design, began with a starting dose of 0.02mg/kg. IMGN779 was administered intravenously once every 2 weeks on days 1 and 15 as part of a 28-day cycle. Adverse events (AEs) were evaluated using NCI-CTC v4.03.

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.

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 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 (39%; G3/4 4%), decreased appetite (35%; G3/4 4%), nausea (30%; G3/4 4%), asthenia (26%; G3/4 4%) and neutropenia (22%). The only Grade 3/4 AE reported in >10% of pts was neutropenia (22%). Two pts had a TGR-1202 dose reduction. Nineteen pts (11 DLBCL/8 FL) were evaluable for efficacy: ORR amongst all pts was 79% (15/19) with 42% (8/19) achieving a complete response (CR), of which 5 were DLBCL and 3 FL. ORR in the respective groups as follows:

<table>
<thead>
<tr>
<th>Group</th>
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Median follow-up time on study is 6 mos for all pts (range 1-14+ mos).

Summary/Conclusions: The combination of UTX, TGR-1202, and bendamustine has exhibited manageable toxicity with significant activity in advanced DLBCL and FL pts including an encouraging 42% CR rate (45% in DLBCL and 38% in FL). Enrollment continues at the 800mg TGR-1202 dose level with the use of growth factor prophylaxis. Safety and efficacy data for all pts will be updated at the meeting. Based upon the early activity of the triplet, future registration directed studies are being planned.

Background: Venetoclax (VEN) in patients with relapsed/refractory non-Hodgkin lymphoma (NHL) is a novel agent in pts with lymphoma. The combination of VEN + rituximab (RTX) + bendamustine is tolerable and active in pts w/ rel/ref NHL and is under Phase 3 testing.

Aims: To assess the activity and safety of VEN + RTX + benda in pts with rel/ref NHL.

Methods: A Phase 2 trial of VEN + RTX + bendamustine in pts with rel/ref NHL: Eligible pts had rel/ref indolent or aggressive NHL. Pts with a history of prior NHL were excluded. Pts ≥ 18 yrs, an ECOG PS of ≤2, and an absolute lymphocyte count (ALC) > 1,000/μl were eligible. Cycle 1 treatment delays were reported at the 600mg dose level, which was later expanded and the 800mg TGR-1202 dose is now being evaluated with stricter eligibility criteria to require an ANC of ≥1.0, and the use of growth factor support in cycle 1 is now encouraged. The most common AE’s included diarrhea (39%; G3/4 4%), decreased appetite (35%; G3/4 4%), nausea (30%; G3/4 4%), asthenia (26%; G3/4 4%) and neutropenia (22%). The only Grade 3/4 AE reported in >10% of pts was neutropenia (22%). Two pts had a TGR-1202 dose reduction. Nineteen pts (11 DLBCL/8 FL) were evaluable for efficacy: ORR amongst all pts was 79% (15/19) with 42% (8/19) achieving a complete response (CR), of which 5 were DLBCL and 3 FL. ORR in the respective groups as follows:

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VENETOCLAX (VEN) IN PATIENTS WITH RELAPSED/REFRACTORY NON- HODGKIN LYMPHOMA (NHL)

1Dana-Farber Cancer Institute, Boston, United States, 2Royal Melbourne Hospital, Parkville VIC, 3Peter MacCallum Cancer Centre, East Melbourne VIC, 4University of Texas MD Anderson Cancer Center, Houston, 5University of Arizona Cancer center, Tucson, Australia, 6AbbVie, Inc., North Chicago, 7Memorial Sloan-Kettering Cancer Center, New York City, United States
Background: 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). Only acceptable toxicity, in dose cohorts ranging from 300-1200mg. Adverse events (AEs) were assessed by NCI-CTCAE v4.0 and response by 2007 Cheson IWG 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 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 treatment was 13 mo (0.2-493). ORR was 75%, 6 pts (21%) achieved CR and remain on study (DORs: 25-40 mo). One pt with a PR proceeded to elective allogeneic stem cell transplant and remained disease free at last protocol defined follow-up (24 mo after coming off study). Median PFS was 11 mo and DOR was 15 mo. MZL pts (median age: 63 years) had received a median of 4 (2-6) prior tx. Time from start of prior tx to start of VEN was 8, 14, 73 mo and time on VEN was 5, 1, 35 mo. One pt (6 prior tx) received VEN for <1 mo due to progressive cytopenias; 1 pt (4 prior tx) achieved a PR with VEN at wk 6 but had PD at wk 16; 1 pt (2 prior tx) achieved PR at wk 6 and is the only pt to remain on study (DOR: 32 mo). WM pts (median age: 67 years) had a median of 4 (3-5) prior tx. Time from start of prior tx to start of VEN was 5, 18, 33, 67 mo and time on VEN was 42, 17, 54, 20 mo. All pts achieved PR (at wks 6 [n=26], 16 and 36), with DORS of 11, 12, 38 and 50+ mo (latter is ongoing and remains on study).

Summary/Conclusions: VEN monotherapy has a tolerable safety profile in MCL, MZL and WM pts. ORR were high and most responses durable; median PFS and DOR suggest significant activity in MCL pts. Further investigation of VEN in each disease is indicated.

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WHOLE BODY DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING IS A GOOD PREDICTOR FOR TREATMENT OUTCOME AFTER ONE CYCLE OF IMMUNOCHEMOTHERAPY IN AGGRESSIVE LYMPHOMA

K. De Paepe 1, F. De Keyser 1, C.-A. Van Keerberghen 2, O. Gheysens 2, V. Vandecaveye 1

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 as an radiation-free imaging technique to predict treatment outcome in NHL after one cycle of ICT (2-3 weeks).

Results: Median follow-up time was 43 months (4-70 months). Thirty-three patients achieved complete remission (CR), 4 progressed 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 b1000mean increase of less than 6% in extranodal lesions. WB-DWI/MRI predicted DFS correctly in 45/46 (96%) [p<0.001; hazard ratio (HR): 0.24, 95% CI 0.05-1.15], and interim FDG-PET/CT was additive to baseline and interim scan (Δr). Statistical analysis consisted of Kaplan-Meier survival analysis univariate and multivariate Cox regression analysis with disease-free-survival (DFS) as outcome measure.

Summary/Conclusions: In the largest single-center, real-world experience of Ibrutinib (IBR) use in DLBCL, RT and FL patients consecutively treated with IBR. Data collected included patient demographics, stage, IPI, genetic characteristics, prior treatments, IBR dose and discontinuation reasons for discontinuation and response, PFS and OS were estimated using the Kaplan Meier method and survival analysis by the log rank (LR) test.

Results: 44 patients were identified (DLBCL: n=24, 54.5%, FL: n=12, 27%, RT: n=8, 18%) who received IBR therapy in the RR setting. Baseline characteristics included age (range 19–80), 61% male, 95% ECOG 0 - 1, 71% stage IV, 62% elevated LDH, and 48% R-IPI ≥ 4. DLBCL sub-types (Hans criteria) were 25% non-GC (n=11), 16% GC (n=7), and 14% unclassifiable (n=6). In the FL subgroup, 8% were grade 1, 58% were grade 2, 33% were grade 3a. Median number of prior therapies was 5 (range 1-11). All RT patients were not treated with IBR previously for CLL. The three most common reasons for IBR discontinuation were progression (35%), toxicity (20%), and bridge to CAR-T (10%). PFS and OS data are shown in Table 1. In DLBCL, cell of origin (IHC) did not impact outcomes [p=0.97, LR test]. Patients with RT had better PFS as compared to de novo DLBCL (p=0.03, LR test).

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Summary/Conclusions: Ibrutinib (IBR), a Bruton’s Tyrosine Kinase (BTK) inhibitor, is FDA approved for chronic lymphocytic leukemia, Waldenström macroglobulinemia, marginal zone lymphoma and mantle cell lymphoma. Despite its limited data, IBR is increasingly being utilized as a treatment option for patients with relapsed/refractory (RR) diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL).

Aims: To 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 discontinuation reasons for discontinuation and response. PFS and OS were estimated using the Kaplan Meier method and survival analysis by the log rank (LR) test.

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and other extranodal localizations (12%). In patients harboring a MYD88 mutation, increased at IP sites (67%, Chi-square P=0.001), compared to nodal (13%) translocations, Epstein Bar Virus (EBV) status and the mutational status of MYD88 and/or CD79B mutations were identified in 51 patients and 19 cases, respectively. CD79B mutations were identified in 32 and 69% of 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.

**Background:** Activating mutations in CD79B and MYD88 are important molecular drivers of a subset of diffuse large B-cell lymphomas (DLBCLs), activating the B-cell receptor and toll-like receptor pathways, respectively. Interestingly, the frequency of these mutations differs greatly among DLBCLs at different anatomical sites, with a remarkably high prevalence at immune-privileged (IP) sites (central nervous system and testis). Recent studies suggest that these mutations are associated with an unfavorable prognosis. However, the prognostic value in relation to the site of presentation has not yet been explored.

**Aims:** To investigate if mutations in MYD88 and CD79B are independent prognosticators for overall survival (OS) in DLBCL, particularly in patients with lymphomas at IP sites, for which a high prevalence of these mutations was reported.

**Methods:** In this retrospective study, we investigated a large clinically annotated cohort of 189 consecutive primary DLBCLs, including primarily nodal (N=64), primarily extranodal (N=74) and IP localizations (N=51). Patients were diagnosed between 1990-2015 at the Academic Medical Center, (University of Amsterdam) or other Dutch hospitals. The vast majority was treated with (R-)CHOP (N=143) or other immune-chemotherapies (N=16). Detailed clinical characteristics of all patients were collected. For all patients BCL2, BCL6, and MYC translocations, Epstein Bar Virus (EBV) status and the mutational status of MYD88 and CD79 were assessed, employing methods described previously (Kraan et al., BCJ 2013).

**Results:** Translocations in BCL2, BCL6 and MYC were identified in 14, 32 and 13 patients, respectively and 23 EBV-positive cases were found. MYD88 and 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=0.01, 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.) identified MYD88 and CD79B as independent prognostic parameters with Hazard ratios 1.8, 1.5 and 2.6, respectively (95% Confidence intervals: 1.0-3.6, 1.0-2.8 and 1.4-3.9, respectively).

**Conclusion:** 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. 

**P568**

**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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1University of Cologne, Cologne, 2Hematology/Oncology, Rotkreuzklinikum München GmbH, München, 3University of Frankfurt, Frankfurt, 4University of Bonn, Bonn, 5University of Munich, Munich, 6University of Essen, Essen, 7University of Düsseldorf, Düsseldorf, 8Vivantes Auguste-Viktoria Hospital, Berlin, 9ICH Study Center, Hamburg, Germany

**Background:** The outcome of HIV-associated lymphoma has undergone significant improvement in recent years beginning with the widespread use of combination antiretroviral therapy (ART). However, among AIDS-related deaths, non-Hodgkin lymphoma (NHL) is the most frequent event. HIV-positive patients (pts) with relapsed NHL or Hodgkin lymphoma (HL) should be treated in a manner similar to immunocompetent pts.

**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-infected pts with biopsy or cytologically proven HIV-related lymphoma diagnosed at 32 participating centers in Germany and Austria since January 2005. Data on HIV-infection and lymphoma characteristics, treatments and outcomes were recorded. Pts with indolent lymphomas and primary central nervous system lymphomas were excluded from the present analysis.

**Results:** Of 499 pts (463 males, 36 females) 394 had aggressive NHL and 105 HL. The median age at lymphoma diagnosis was 45.6 yrs (range, 22–74). 344 pts (69%) were diagnosed with advanced stage (III/IV) lymphoma and the median CD4-cell count was 271/µl (266/µl in NHL and 287/µl in HL). 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 14-1,4-41.3 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.
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 scans, no systemic treatment, uncertain histology, and post-treatment 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.

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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.

Figure 1.
Summary/Conclusions: Crizotinib confirmed to be an effective and safe therapy for advanced relapsed ALK+ ALCL with durable responses up to 6 years after treatment initiation and no relapse later than 4 months. These results represent the longest available safety record for crizotinib. ALK point mutations can develop and 2nd/3rd generation inhibitors may be a therapeutic opportunity for patients who develop resistance to crizotinib.

P571 PRELIMINARY RESULTS FROM AN OPEN-LABEL, PHASE II STUDY OF TIPIFARNIB IN RELAPSED OR REFRACTORY PERIPHERAL T-CELL LYMPHOMA

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Background: Tipifarnib is a potent and selective inhibitor of farnesyltransferase (FT). FT catalyzes post-translational attachment of farnesyl groups required for 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 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 AEs (grade ≥ 3) were myelosuppression, including neutropenia (61%), anemia (39%) and thrombocytopenia (39%). 3 pts achieved a partial response (2 AITL; 1 PTCL-NOS) and 3 additional pts experienced stable disease >6 months. Tumor DNA from 18 pts was sequenced using NGS/CGP. 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) 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 high levels of CXCL12 gene expression.

22nd Congress of the European Hematology Association

P572 BAMB CONDITIONING BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION FOR LYMPHOMA: A RETROSPECTIVE STUDY ON BEHALF OF THE BRANCHOPHONE SOCIETY OF BONE MARROW TRANSPLANTATION AND AUTOLOGOUS STEM CELL THERAPY (BAST) GROUP

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Background: High-dose chemotherapy before autologous stem cell transplantation (ASCT) is a therapeutic option as a consolidation in primary or relapsed lymphoma. BEAM conditioning is generally used. Alternative conditioning regimens have been published but few data are available.

Aims: To evaluate tolerance and efficacy of the BAM (Busulfan, AraCytin and Melphalan) conditioning before ASCT.

Methods: We conducted a retrospective study in 188 French patients treated between 2000 and 2015. Data were retrospectively collected from the Promise database. Informed consent was obtained from all patients.

Results: Indications for ASCT were diffuse large B-cell lymphoma (n=54, 29%), mantle-cell lymphoma (n=42, 22%), Hodgkin’s disease (n=33, 18%), low-grade non-hodgkin lymphoma (n=26, 14%), T-cell lymphoma (n=17, 9%), Burkitt’s lymphoma (n=8, 4%) and B-cell lymphoma (n=8, 4%). Median age at diagnosis was 50.9 years (35.7-59.9). 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 rituximab therapy. In all patients, ASCT was the first transplantation. In 11 patients, ASCT was planned as part of a multiple graft protocol. At the time of transplantation, 116 (62%) patients were in complete remission, 54 (29%) in partial remission, 13 (7%) in relapse or progression, and 5 (2%) ND. ASCT was documented in 186 (99%) patients. Median time to neutrophil and platelet (>50 GigalL 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 undocmented fever, 19 (10%) had sepsis, 150 (80%) had grade 1-4 mucositis during neutropenia with a WHO toxicity grading of 2 (42%), 3 (39%) and 4 (19%). Colitis with a median duration of 7 days [5-10], was reported in 73 patients, with a maximum toxicity grading of 1-2 (n=43, 59%), 3 (n=21, 29%) or 4 (n=4, 6%) and ND in 5 patients. Only 2 (1%) patients had non-fatal hepatic sinusoidal obstruction syndrome. Pulmonary toxicity was reported in 33 (17.6%) patients with 8 cases of respiratory distress syndrome. Respiratory distress was fatal in one patient but occurred more than 6 months after ASCT and salvage treatment. Seven (3.7%) patients 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-NR]. 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.

Conclusion: 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 MICRONAAOME, PROTEOME AND METABOLOME OF EXOSOMES FROM PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA
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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a clonal disease caused by the lack of glycosyl inositol phosphatidyl anchored proteins at the cell membrane that lead to intravascular hemolysis upon complement activation. Patients have intravascular haemolysis with high risk of thrombosis, and a variable degree of bone marrow failure. Treatment with Eculizumab reduces intravascular hemolysis and also the thrombotic risk. The mechanism of thrombosis in PNH is still unknown. Exosomes are small 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 expression was evaluated by plasma/serum focus miRNAs PCR panel V4 (Exiqon). Proteomic analysis of exosomes was performed at the OMCs 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 unequivogue structural elucidation of unknown metabolites.

Results: Mir-16-5p and miR-451a had lower levels in patients vs controls. Eculizumab treatment increased their expression, particularly in the group with thrombosis. Eculizumab also decreased mir-223-3p (the most abundant miR) 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 V4 region HG3 increased in 3.9-fold in the Eculizumab group vs without Eculizumab group, which could be related with the treatment. We identified quite few metabolites inside the exosomes, all of them associated with cell toxicity or immune response. The levels of Cholesterol, HydroxyTertbinaine-glucuronide and Diacylglycerol 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 suggests that exosomes contain material that may influence the pathologic 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: THE ROLE OF PRIMARY IMMUNODEFICIENCY AS CAUSATIVE AGENTS. A SINGLE CENTER DATA
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Background: Severe Chronic Neutropenia may be a primary disease, usually defined as congenital (CN), or a condition mainly secondary to autoimmune disturbances (SN) (1,2). CN rises in early infancy, has a narrow block at pro/myelocyte, classically carries genes ELANE/HAX1 mutations in 70% of cases and is G-CSF dependent. SN is accompanied by extraheamatological 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 either group and share features of both of them. These “Overlap Neutropenia” (ON) patients are a diagnostic and management challenge.

Aims: Investigate the genetic background of this ON from a cohort of chronic neutropenia subjects screened at Hematology Unit of Gaslini Hospital and characterize their clinical phenotype.

Methods: Patients with severe chronic neutropenia were seen prospectively in our center and diagnosed/followed-up according to published guidelines(3,4).

Genetic diagnosis includes classical Sanger technique foe commonest severe chronic neutropenia genes and an enlarged NGS panel including also those genes responsible for PID.

Results: From 2008 to 2016, 24 patients (13 males) with median age at last follow of 18yrs (range 20 mo-51y) had a complete work up for severe chronic neutropenia (Table 1). Ten/24 subjects (43%) were diagnosed as classical CN with ELANE mutation found in the majority (80%) of cases. Seven/24 (29%) were diagnosed as SN and the remaining 7/24 (29%) as ON. A PID genes mutation was found in a total of 8/24 patients (30%) with 5 patients belonging to 7 SN subjects (71%) and 3 to the 7 ON subjects (42%).Table 1 shows clinical hematological characteristic of the 3 categories of patients.

Summary/Conclusions: A considerable portion (30%) of subjects affected with severe chronic neutropenia have been identified as PID. In the group of ON subjects a mutated PID gene was found in 3/7 patients and mutations of ELANEin 2/7 patients. No mutation was found in the remaining 2. The phenotype of ON subjects is characterized by extra-heematological 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.

TREATMENT WITH HORSE-DERIVED ANTI-THYMOCYTE GLOBULIN LENDS 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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To evaluate the safety and efficacy of first line treatment using ATGAM and ciclosporin in adult patients with acquired aplastic anemia in the Netherlands.

Methods: Data on adults with acquired aplastic anemia who received ATGAM as first-line treatment was collected in the LUMC, AMC, UMCG, UMCU, VUMC, MC, UMCN, Erasmus MC, Medisch spectrum Twente and Antonius Nieuwbeek Nieuwegein. The data included baseline-characteristics and follow-up data at 3, 6, 9 and 12 months. After 12 months, follow-up data was collected at least yearly. All patients received first-line treatment with ATGAM (40mg/kg for 4 days) and ciclosporin. Response was defined as complete in case of normalization of blood values and as partial in case of transfusion dependency 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 NVvH registry. Median 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. After initial response at 6 months, aplastic anemia relapsed in 4 patients and 1 patient developed AML. From the 23 non-responding patients, 7 patients continued ciclosporin after 6 months without additional treatment. Five of these 7 patients became transfusion independent up to 18 months after treatment with ATGAM. Sixteen of the patients without a hematological response 6 months after ATGAM received second-line treatment consisting of alloSCT (n=8), Thymoglobulin (n=4), Eltrombopag (n=3) or Danazol (n=1). From the 16 patients that received second-line treatment, 10 patients eventually had a hematological response. Patients without a response at 6 months, had an overall survival probability 12 months thereafter of 90%. One patient developed secondary AML after second-line treatment with Thymoglobulin.

Summary/Conclusions: 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.

P578 DEVELOPMENT OF A SCREENING AND DIAGNOSTIC ALGORITHM FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA USING A MODIFIED DELPHI PANEL METHODOLOGY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoietic stem cell disorder that manifests with hemolytic anemia due to uncontrolled complement activation, bone marrow failure, and thrombosis. Diagnosis is essential because PNH is a progressive disorder associated with substantial morbidity and mortality. The protean clinical manifestations of PNH complicate diagnosis, and subsequently the diagnosis is often delayed or missed. Although national diagnostic guidelines are available, international expert consensus on PNH screening and diagnosis is lacking.

Aims: An international panel of PNH experts was assembled to develop a clinically relevant, consensus-driven screening and diagnostic algorithm for PNH.

Methods: An expert advisory committee of 4 PNH experts from North America, Europe, and Japan was assembled. Using a modified Delphi methodology, consensus was gained on the symptoms and signs of PNH and the laboratory tests required for screening and confirmation of diagnosis. Globally representative Delphi panelists were identified through a double-blinded screening process and asked to complete 2 rounds of web-based questionnaires. The questionnaires were developed by the expert advisory committee and presented to the Delphi panel in a case-based format. In the first round, Delphi panelists were given 5 blinded case studies—each including details on clinical presen-
tation and past medical history—and were asked to provide their differential diagnosis and the tests they would order to establish the diagnosis in free-text format. To reduce bias, Delphi panelists were blinded to the fact that the study was focused on PNH. Responses mentioned by ≥50% of Delphi panelists in the first round were included in the second-round questionnaire. For each case in the second-round questionnaire, Delphi panelists were presented with a set of consensus statements regarding potential diagnoses and the need for specific tests/data from a multiple-choice list and asked to respond with their level of agreement on a 4-point Likert scale. Consensus in the second round was attained if ≥80% of Delphi panelists agreed on a given screening or diagnostic approach.

Results: Twelve Delphi panelists from 6 countries, all of whom were clinicians with expertise in PNH, were recruited. Consensus was reached on 22 of 23 PNH screening and diagnostic decision points identified by the Delphi panelists. Specifically, consensus was gained on the core symptoms and signs of PNH at presentation, including hemolysis, bone marrow dysfunction, and thrombosis. Consensus was reached on 36 of 38 questions in the second round, and the tests required at each decision point to narrow the differential diagnosis and to confirm the diagnosis of PNH. The level of agreement on screening and diagnostic decision points and tests was sufficient to enable the development of a screening and diagnostic algorithm (Figure) that is consistent with the published literature and with the real-world experience of the international expert advisory committee.

Summary/Conclusions: The modified Delphi methodology facilitated development of a consensus-based, clinically relevant PNH screening and diagnostic algorithm. This algorithm provides clinicians with varying levels of expertise detailed guidance on how to screen for and diagnose PNH.

P579

DIAMOND-BLACKFAN ANEMIA IN THE NETHERLANDS: AN OVERVIEW OF CLINICAL CHARACTERISTICS AND UNDERLYING MOLECULAR DEFECTS

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Background: Diamond-Blackfan anemia (DBA) is a rare genetic disorder, characterized by bone marrow failure (anemia), congenital anomalies and a predisposition for malignancies. DBA is characterized by a highly heterogeneous nature, both clinically and genetically. Most of our understanding of this disorder stems from molecular studies combined with extensive data-input from international patient registries.

Aims: The aim of our retrospective study was to create an overview of the pediatric DBA population in the Netherlands.

Methods: Forty-four patients (age 0-18yr) diagnosed with DBA from all Dutch pediatric centers included in this study. Clinical and genetic characteristics were collected from patient records.

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 variant, described in a patient with multiple congenital abnormalities (craniofacial defects, cardiac defects, colitis) in addition to severe anemia. Thirty-four (3/44) patients were treated with glucocorticoids, of which in thirty-one (31/34) patients a complete response was observed (91,2%). However, in 29% discontinuation was prompted by high-dose dependence, side effects, a weaning response, or a combination of these factors. Five patients (12,2%) were successfully transplanted with hematopoietic stem cells from either matched sibling donors (n=3) or matched unrelated donors (n=2), including two cases after the age of 10 years. Eleven patients (26,8%) were treatment-independent, defined as acceptable hematological parameters 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 conclusion, we have identified a novel variant 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 integrated with multi-colour flow panels including CD61 and FLAER. The criteria for determining discordant granulocyte and monocyte clone sizes was dependant upon the absolute size of the smaller clone. For clones 0.1-10%, discordance was defined as when the larger clone was either ≥2 x the smaller clone or at least 1% (absolute value) greater. For smaller clones >10%, the larger clone had to be ≥110% its size. IST was uniform - Cyclosporine (CSA, 2.5mg/kg p.o. b.i.d.), anti-thymocyte globulin (ATG; ATGAM® 40mg/kg IV daily x 4 days) and (Methylprednisolone 1mg/kg/day x 10 days). CSA doses were adjusted to maintain whole blood trough CSA level of 200-300 ng/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); 30 pts remain alive and well with a median follow-up of 48 mos (15-86). There were 9 pts (CNS 0.1-10%), the larger clone had granulocyte-dominant disease (Group 2) and 9 pts (≥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 BW) and prednisone (day 1-29).

Results:

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/17</td>
<td>2/9</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>

Response rate (%): 12/17 (70.6%) 4/4 (100%) 4/4 (100%) 0.096.

Summary/Conclusions: Flow cytometry for a PNH clone is routinely done in AA although it may be important to evaluates both granulocyte and monocyte clone sizes. Pts with a larger monocyte than granulocyte clone size more frequently have NSAA and appear to have a lower response rate to IST. This may have therapeutic implications and could identify a population of pts requiring a unique therapeutic approach.

P582

RESPONSE TO ANTI-THYMOCYTE GLOBULIN (ATG) IN PATIENTS WITH APLASTIC ANEMIA (AA): A SINGLE-CENTRE EXPERIENCE OVER THE LAST 28 YEARS

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Background: Aplastic anemia (AA) is a rare, usually acquired disorder characterized by bone marrow failure with bi- or pancytopenia and marrow hypoplasia. The classification into the three main subtypes is of prognostic and therapeutic relevance. Depending on disease severity, patient’s age, and the availability of a potential HLA-identical donor, different therapeutic strategies arefavorable immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporine (CsA) is considered as 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 of Essen between 1988 until 2015, 30 pts were evaluated and monitored at the Department of Hematology between 1988 until 2015. 73% of the pts (49/67) were treated with hATG (ATGAM® (44/49) vs >50 yrs: 43% (13/30); p<0.001). In primary refractory pts (34% (23/67)) (52% (12/23) in first-line treated hATG pts vs 48% (11/23) rATG treated pts) a second course with either hATG (3/9) or rATG (6/9) was initiated, achieving an overall hematologic recovery at 6 months in 3 pts (33% (1/3) hATG vs 33% (2/6) rATG treated pts). A disease relapse (median: 13 months after primary ATG therapy) was seen in 11 out of the 44 pts with primary hematologic recovery (25%) (82% (9/11) in first-line treated hATG pts vs two rATG treated pts). A salvage therapy with rATG was initiated in two pts, whereas in one other pt a second course with hATG was started. An overall response following relapse therapy was observed in 33% of the pts (1/3). Four refractory as well as relapsed pts were treated with eltrombopag respectively (final results are still awaited). A secondary HSCT (hematopoietic stem cell transplantation) was performed in 11 out of the 67 pts (16%), either being primary refractory or due to a disease relapse.

Summary/Conclusions: Our data are able to independently confirm the findings of previous studies concerning hematologic recovery rates in pts with acquired AA following IST with ATG by providing further evidence that ATG plus CsA is inferior to hATG plus CsA when administered as a first-line treatment. In addition, we were able to observe in pts ≤50 yrs, irrespective gender, an overall higher hematologic recovery. For this reason, it remains unclear why ATGAM® is still not approved in Germany as first-line therapy in pts with AA, as the only hATG product registered in Europe (Lymphoglobulin®) was withdrawn from the market in 2007.

Table 1.
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 higher frequency in unmutated IGHV (IGHV-UM) immuno-chemorefractory 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 a one-color labeling strategy using a one-color labeling strategy using the 4x44K platform. Specific gene/protein validations were performed by QRT-PCR, western blotting, flow cytometry and immunofluorescence. CLL-like MEC-1 cell line was transfected with a vector containing a NOTCH1 intracellular domain (NICD) or with a control vector. Cell proliferation was evaluated by CellTrace assay. Cell size was estimated by flow cytometry from Forward Scatter (FSC) values.

Results: i) A GEP comparing purified cells of 10 IGHV-UM CLL cases (5 NOTCH1-mut; 15%>37% of NOTCH1 mutated alleles) selected nucleophosmin-1 (NPM1) and genes regulating for several ribosomes as significantly up regulated in NOTCH1-mut cases. Agher 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 IGHV UM subgroup. ii) Western blotting in 11 CLL cases (5 NOTCH1-mut) confirmed a higher NPM1 protein expression in NOTCH1-mut cases, with a direct correlation with NOTCH1 expression (r=0.814). In NOTCH1-mut cases, the NPM1high subpopulation, isolated by cell sorting, showed a higher NOTCH1 mutational 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 effects were confirmed by co-culture of CLL cells with the JAGGED1-expressing M2-10B4 stromal cells. Inhibition of NOTCH1 signaling by gamma-secretase-inhibitor L685,458 or by siRNA for NOTCH1 reduced NPM1 expression (Fig. A). iv) Previous studies identified MYC as a direct transcriptional target of NOTCH1 (Brock et al, Genes and Development, 2008; Andritsos et al, PNAS 2009) and, in turn, a transcriptional activator of 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 48 h culture, NOTCH1-mut CLL cases showed increased MYC transcript levels than NOTCH1-wt cases. MYC expression was further increased upon NOTCH1 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. v) NPM1 silencing by siRNA was able to reduce proliferation rates and cell size of both NICO-transfected cells and control cells. In keeping with a NOTCH1-driven regulation of cell growth/ proliferation and 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 PERsist AT INCREASED COUNTS AFTER SEVEN YEARS OF FOLLOW-UP

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Background: The presence of very low numbers of clonal B cells in peripheral blood (PB) of otherwise healthy individuals (low-count monoclonal B lymphocytosis-MBLlo) is a common finding in the general population (20-30% of individuals). The 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 1 B-cell 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 [del13q14.3(D13S25), trisomy 12, del(11q(ATM)) and del(17q(TP53))] and del(1q) were studied at baseline and at 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 4 fold overall increase in the number of B-cell clones after a 7y 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 showed a size increase and a change in cytogenetic alterations; among those, del(13q) was the most common abnormality 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 (13/36) and del(17q)(TP53) (1/36). A statistical association (p=0.05) was found between the overall increase over time in the size of these clones and the presence of cytogenetic lesions. Three subjects developed lymphocytosis (median: 5.3x10^9 lymphocytes/l; range: 4.1x10^9-5.9x10^9) after 7 years; in these cases the clone size increased sub-
MICROENVIRONMENT REGULATION OF PROGRAMMED DEATH-1 (PD1) RECEPTOR AND ITS LIGANDS PD1 AND PD2 IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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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 organization and gene regulation. In particular, Lamina Associated Domains (LADs) were identified at the nuclear periphery as transcriptionally silent, gene-poor domains of the genome. Lamin B1. More recent studies however revealed an important role of LADs in the regulation of gene expression and recombination. Aims: Given the apparent topological coincidence between LADs and Ig variable clusters, we hypothesised that nuclear lamina might play a paramount role in the dynamics of Ig-encoding variable genome domains. In particular, here we tested whether Lamin B1, a principal LAD-associated component of the nuclear envelope, had any restrictive role on somatic hypermutation (SHM) and the expression of Ig genes. Due to the strong involvement of IgV mutations in the pathogenesis of B-cell malignancies, we also tested whether nuclear lamina is involved in the pathogenesis of 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 resting and activated BL2 and B cells. LMNB1 RNAi was used to obtain the somatic hypermutation. ChIP-Seq, ChIP-PCR and ImageStream analyses were performed to establish Lamin B1 suppressive environment when SHM was induced during B cell activation and formation of lymphoid germinal centres. ChIP-Seq and the expression of Ig genes. Due to the strong involvement of IgV mutations in the pathogenesis of B-cell malignancies, we also tested whether nuclear lamina is involved in the pathogenesis of germinal centre lymphomas and chronic lymphocytic leukaemia (CLL).

Results: We have found that genome binding of Lamin B1, a component of the nuclear envelope involved in epigenetic chromatin regulation, is reduced during B cell activation and formation of lymphoid germinal centres. ChiP-Seq analysis of BL2 and heavy and very heavy immunoglobulin domains were released from the Lamin B1 suppressive environment when SHM was induced in B cells. RNAi-mediated reduction of Lamin B1 resulted in spontaneous SHM as well as kappa-light chain aberrant surface expression. Finally, Lamin B1 expression level correlated with progression-free and overall survival in chronic lymphocytic leukemia, and was strongly involved in transformation of follicular lymphoma.

Summary/Conclusions: In summary, here we report that Lamin B1 is a negative epigenetic regulator of SHM in normal B-cells and a “mutational gatekeeper”, suppressing the aberrant mutations that drive lymphoid 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. 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.

Methods: Eighteen primary CLL samples were treated with IL-4 +/- ibrutinib (1μM) and expression of FOXP1, GAB1, PTEN, SOCS1 and SOCS3 assessed by immunoblotting. The effect of ibrutinib on apoptosis was assessed by flow cytometry and PI/Annexin V staining.

Results: Primary human CLL cells treated with IL-4 for 24 hr significantly increased expression of positive regulators of BCR signalling FOXP1 and GAB1 in CLL samples with un-mutated IGHV (U-CLL); no change in expression in FOXP1 or GAB1 was seen in CLL samples with mutated IGHV (M-CLL). There was a 40% increase in PTPN22 expression in IL-4-treated U-CLL samples vs no change in M-CLL. ibrutinib, at therapeutic concentrations, blocked IL-4 mediated decreases in FOXP1, GAB1 and PTPN22 and pSTAT6 (a positive control for IL-4 signalling). After 24 hr 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 ibrutinib. CLL cells with expressed VLA-4 and FOXP1 could be induced apoptosis 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 ibrutinib can overcome IL-4/CD40L induced expression of pro-survival proteins MCL1 and BCLXL and that cedulatin in combination with venetoclax can induce apoptosis in a synergistic manner in the presence of IL-4/CD40L. We now extend these results to assess the importance of this drug combination in the presence of BCR stimulation. The combination of ced and venetoclax in the presence of either BCR signalling (bead immobilised anti-IgM) alone, or combined with IL-4 and CD40L, induced synergistic killing, with greater CLL cell death than with either drug alone.

Summary/Conclusions: These results provide evidence that IL-4 may increase BCR signalling by upregulating the expression of positive regulators of BCR signalling in U-CLL and that this can be overcome by ibrutinib. These results support the continued use of ced in clinical trials for the treatment of CLL, alone or in possible combination with venetoclax.
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 IcI CLL study (ISRCTN12695354).

Aims: The IcI CLL 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 (420 mg 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, 9 & 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. 1×10⁶ leukocytes were tagged to extracellular antibodies (CD3/CD19) conjugated to fluorochromes. Ibrutinib (10μM) was added to the cells for 30 minutes at 37°C followed by anti-IgM/IgD stimulation (10μg/ml). The BD phosphoflow protocol was followed to lyse/fix/permeate the CLL cells. Antibodies to Btk pY551, Syk pY348, ERK1/2 pT204/pY204, Akt pS473 were used tagged to fluorochromes (from BD Biosciences). Cells were acquired on a BD Fortessa flow cytometer.

Results: The phosphorylation of Btk, Syk, Akt and ERK1/2 was analysed in cells at the specified time points and conditions for 20 TN and 20 RR CLL patients. Baseline phosphorylation of all kinases was similar in both PB & BM. Phospho-Btk showed no stimulation on addition of IgM/IgD 4h after initiating therapy. There was a strong (2-4 fold) increase in phosphorylation of Syk kinase with IgM/IgD even in the presence of ibrutinib in vitro. This effect was profound in the first 2 months of ibrutinib therapy with a general decrease in phosphorylation after 6 months. Baseline stimulation of ERK1/2 gave a 1.5-2 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 P38 kinase inhibitor may result in complete BCR blockade. Phosphorylation patterns may also act as an adjunct to ascertain the response to therapy.

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EVALUATION OF COMBINATIONAL THERAPIES FOR RELAPSED/REFRACTORY CLL WITH MUTATED P53
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Background: Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with survival ranging from months to decades. CLL patients harboring TP53 alterations are well known to be refractory to standard therapies; however, recent studies indicate that ibrutinib, a Bruton’s tyrosine kinase (Btk) inhibitor, suppresses the B-cell receptor (BCR) signaling pathway and is an effective treatment option for these patients. Unfortunately, many patients with TP53 alterations will ultimately fail ibrutinib-based therapies. Similarly, we have used a mouse model of refractory p53 mutant CLL (Eμ-TCL1;p53R172H) and reported that while ibrutinib is effective in reducing the CD5+CD19+ population and extending survival, these mice eventually succumb to the disease (Lee HJ, BCU 2016). These incomplete therapeutic responses indicate that ibrutinib provides only a temporary respite for this refractory disease, and highlights our need to develop more potent and targeted combinations.

Aims: Ibrutinib is effective in delaying (but not eliminating) leukemic progression in p53 mutant CLL, suggesting that combinational therapies that inhibit BCR signaling and activate apoptotic programs may be effective therapeutic strategies. Thus, agents that do not require activation of p53 but are effective in blocking oncogenic pathways (BTK and BCL-2) are attractive options. Currently, ibrutinib and ABT-199 meet this criteria and thus, we hypothesize that simultaneous inhibition of the BTK- and BCL-2-pathways will be an effective strategy in treating p53 mutated CLL.

Methods: To test this, we used RNA-Seq to examine expression changes in B-cells from Eμ-TCL1 mice carrying either wild type or a single p53R172H hotspot mutation (corresponding to p53R172H in humans) following ibrutinib treatment. qRT-PCR and IHC were used to validate expression of key targets within pathways amenable to combinational therapy. Hematopoietic tissues were subjected to combinational therapies to interrogate efficacy.

Results: We have shown that ibrutinib downregulates the BTK- and ERK-pathways regardless of p53 status. However, less is known in regards to global expression changes in p53 mutant CLL following BTK inhibition. To investigate this, we performed RNA-Seq analyses using malignant B-cells from untreated and ibrutinib treated Eμ-TCL1;p53R172H and Eμ-TCL1 mice. Pathway analyses revealed that CLL cells harboring a single p53 mutant allele retained a partial ability to activate p53-dependent programs. qRT-PCR revealed robust activation of p53-dependent anti-proliferative targets like p21, but only modest activation of pro-apoptotic targets (e.g.; PUMA), suggesting these p53 mutant CLL cells have diminished capacity to activate apoptosis or overcome apoptotic inhibitors. To explore this altered bi-modal p53 activation, we performed IHC and observed that apoptotic activation was hampered by increased BCL-2 expression. To examine whether this BCL-2-dependent inhibition could be overcome, malignant B-cells were treated with ibrutinib alone, ABT-199 (a BCL-2 antagonist), or in combination. Here, we observed that ABT-199 was sufficient to activate apoptosis, regardless of p53 status, and that its use in combination with ibrutinib drastically reduced cell viability.

Summary/Conclusions: Together, these data indicate that patients with a partially attenuated p53 pathway may retain the ability to activate apoptosis if molecular barriers are removed (e.g.; BCL-2 via ABT-199). Furthermore, these results suggest that combinations with BTK- and BCL-2 inhibitors may be therapeutically beneficial for patients with mutated TP53.
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THE DNA REPLICATION PATHWAY HAS POTENTIAL PREDICTIVE VALUE FOR TKI RESPONSE AND THERAPEUTIC INTERVENTION IN CHRONIC MYELOID LEUKAEMIA
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Background: Chronic myeloid leukaemia (CML) is a myeloproliferative disease which arises in a haemopoietic stem or multipotent progenitor cell with the t(9;22)(cq34;q11) chromosomal translocation. Tyrosine kinase inhibitors (TKIs) were developed to target the constitutively active oncoprotein BCR-ABL, which is expressed as a result of this translocation. TKI therapy has significantly improved patient survival, however predicting response to therapy is one of the unmet clinical challenges in CML. Moreover, TKIs are unable to target the leukemic stem cells (LSCs) which drive the disease; persistence of LSCs therefore remains a major obstacle to cure CML. Understanding the mechanisms that LSC employ to survive TKI treatment is necessary to design essential therapeutics to eliminate CML in the future.

Aims: To identify genes with predictive value for TKI response and to determine the efficacy of drug targeting one of the key pathways identified.

Methods: Microarray, Fluidigm, Real-time PCR, FACS based cell cycle and Annexin V apoptosis analysis, Trypan blue exclusion cell counts.

Results: Analysis of bulk CML patient microarray data (GSE47927) identified 323 deregulated genes either in the stem cell population or during disease progression. DNA damage responses and 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 signatures 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, 8 and ORC2 in the most primitive LSC (CD34+38−90+53+) compared to CD34+38−90+93− cells. Next, we investigated the ability of heliquinomycin (HQ), a potent helicase inhibitory of MCM on its own and in combination with IM to target the CML cell line K562. Our extensive dose and time response studies followed by FACS-based apoptosis and cell cycle analysis prove the potency of HQ and its synergistic action in combination with imatinib. We also investigated the changes in 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.

Summary/Conclusions: Single cell 'omics' experiments are invaluable 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.

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SIGNAL TRANSDUCING ADAPTOR PROTEIN-1 (STAP-1) MAINTAINS CHRONIC MYELOID LEUKEMIC STEM CELLS
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Background: Signal transducing adapter protein (STAP) 1 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 effectors, including ERK, STAT5, BCL- XL and BCL-2. The family of STAPs includes STAP-1, identified as a c-kit interacting protein, and STAP-2. While STAP-2 is expressed ubiquitously, STAP-1 has hematopoietic-specific expression in mice. It is still unknown whether STAP-1 plays a role in CML, although STAP-1 is expected to have similar functions based on the structural homology between STAP-1 and STAP-2.

Aims: To elucidate the role of STAP-1 in CML using mouse model and human samples.

Methods: We generated STAP-1 deficient mice of the C57BL/6J genetic background. For establishment of CML mouse model, we isolated Lineage (Lin)− Sca-1+ c-kit+ (LSK) fraction of bone marrow (BM) cells from STAP-1+/+ and STAP-1−/− mice, infected them with retrovirus carrying MSCV-BCR-ABL-res-GFP, and transplanted into congenic recipients, that were named Wild type (WT) and STAP-1−/−CML mice, respectively. Human BM samples were collected after informed consent, using protocols approved by the Investigational Review Board of Osaka University Hospital.

Results: Using Western blot and immunoprecipitation assay, we confirmed that STAP-1 binds to BCR-ABL. CML mice model was then employed to analyze the role of STAP-1. We found that STAP-1−/−CML mice showed significantly longer survival than WT CML mice (Fig. 1). STAP-1−/−CML mice displayed less severe splenomegaly and lung hemorrhages compared to WT, suggesting that loss of STAP-1 attenuates CML progression. To investigate how STAP-1 regulates CML progression, we evaluated leukemic stem cells (LSCs) in CML mice. The absolute numbers of STAP-1−/−LSCs (GFP+ LSK) in BM and spleen were significantly lower than those of control (WT vs STAP-1−/−; 2090.3 ± 694.07 cells vs 412.57 ± 114.07 cells in BM, p=0.0291; 12.9 ± 1.75 ×104 cells vs 4.09 ± 0.72 ×104 cells in Spleen, p=0.0009). In colony-forming assay in vitro, STAP-1−/− LSCs generated less colonies in the first and second plating compared to WT LSCs. These data indicated that deletion of STAP-1 would impair self-renewal capacity of LSCs. When we transplanted STAP-1+/+ or STAP-1−/− mice without BCR-ABL transduction in the presence of competing BM cells, deletion of STAP-1 had no effects on engraftment at 28 days after transplantation. Furthermore, we measured the expression of STAP-1 in BM cells derived from patients in the chronic phase of CML. As a result, STAP-1 mRNA was abundant in the LSC (CD34+ CD38−) compartment.

Figure 1.

Summary/Conclusions: In this study, we utilized CML mouse model and showed that STAP-1 is required for progression of CML. Our findings indicate that STAP-1 has an indispensable role in LSC maintenance, while normal hematopoietic stem/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 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...
cell division and telomere length (TL) in peripheral blood cells has been shown to correlate with disease stage, response to treatment and duration of CR 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 retrospective study to compare 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 NCT00852566 study (Nordic CML Study Group) were retrospectively analyzed. All patients studied were available for 14 patients. Of those, 2 (14%) belonged to the Sokol high risk group, 5 (36%) to intermediate and 7 (50%) to the low risk group. CD34+38- cells sorted from bone marrow samples were tested with the standard FISH method using dual fusion dual color BCR-ABL staining following standard procedures. After capturing the BCR-ABL staining using confocal microscopy, samples were re-processed for TL analysis by Q-FISH using established protocols. TL staining was analyzed in all previously captured cells allowing the identification of BCR-ABL+/- cells within the same sample. Analysis and quantification of BCR-ABL FISH staining and TL measurement by Q-FISH were performed in blinded fashion.

Results: Overall, we observed significantly shortened TL in the BCR-ABL+ compared to BCR-ABL- cells (-4.9 arbitrary units (a.u.) range: -53.7 to 16.9 a.u., p=0.04). Next, we correlated the clone size (i.e. the proportion of BCR-ABL+ positive cells within the CD34+38- compartment) with the degree of telomere shortening in LSC. Mean clone size of the patients was 59.9 ± 22.0 % S.D. Of note, we found a significant negative correlation (R=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 CLIP qPCR data R=0.70 (Clonogenic assay by Dr. Corradin) vs Sokol (R=0.40, p=0.03) 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 PREEXISTING SOMATIC MUTATIONS THAT MAY PREDICT PROGRESSION TO BLASTIC PHASE INDEPENDENTLY OF BCR-ABL1 MUTATIONS
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Background: BLastic phase of chronic myeloid leukemia (BP-CML) remains mostly incurable even with newer generation tyrosine kinase inhibitors (TKI) and represents an urgent clinical need. Although in recent years a dramatic reduction in the transformation of chronic phase (CP-CML) to BP-CML has been observed, still up to 5% of patients will progress to BP-CML despite treatment with TKI. Prospective identification of such patients may have a significant clinical impact. There are only few reports to date which use next-generation gene sequencing (NGS) to look for somatic mutations - other than those affecting kinase domain of BCR/ABL 1 - at the time of diagnosis (Dx) which could have a prognostic/predictive value.

Aims: We analyzed the mutational spectrum of somatic mutations in two groups of CML patients with clinically different disease course: first group (BP) comprised of 11 patients who progressed to BP-CML despite treatment with TKI and/or allo-HSCT (one patient) 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 gene database were filtered out and the subsequent analysis was focused on putative protein damaging variants, supported by variant effect prediction tools such as PolyPhen-2, SIFT or CHASM. All reported variants were reconfirmed by Sanger sequencing.

Results: The BP group comprised of paired samples from 11 CML patients who progressed to BP and died despite treatment with TKI. Median age at diagnosis was 53y (range 26 -77), median time to progression for 9 patients (2 were diagnosed in accelerated phase or BP) was 17.5 months (mo) (range 4-108) and median survival was 22 mo (range 10 -116). None of those patients harbored BCR/ABL1 mutation at the time of Dx and progression to BP-CML, 4 patients had additional chromosomal alterations at progression to BP including two frequent (trisomy 8 and monosomy 7). Targeted enrichment followed by NGS allowed us to achieve deep coverage (>80% ge50). Median number of rare variants was 26 (range 18-38) and 29 (range 23-32) for Dx and progression to BP-CML respectively. In total, 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 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 group, ranging between Dx sample and BP sample (range 48-128). In 2 patients (23%, 5%) frameshift mutation in ASXL1 (p.Gly643_Gly644fs) was detected, identical as one of BP patients. Additionally, one patient harbored RUNX1 mutation (p.Arg201Cm) which was not detected in the BP group.

Summary/Conclusions: Our results provide new insights into the already complex genomic landscape of CML. We suggest that a significant number of patients with poor disease outcome may harbor preexisting mutations in DNMT3A, RUNX1 and IDH1. In contrast, mutations in ASXL1 may be present at Dx in patients who will remain in long-term remission.

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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 (IDO) 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 pro-inflammation as well as expansion of immunosuppressive regulatory T cells. Derepression 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 cell surface markers of the IDO-pathway (soluble sIDO1=sIDO1 and kynurenine/tryptophan ratio=kYN/TRP as a product of IDO1 activity) as well as various leukocyte populations such as plasmacytoid dendritic cells (pDC) were analyzed alongside the prospective pan-european ENEST1st clinical study (NCT01061177). This study included 35 CP-CML patients and 26 of them 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: Soluble IDO (sIDO1) levels and KYN/TRP ratio are significantly up-regulated in newly diagnosed CP-CML and drop during nilotinib therapy. sIDO1 levels significantly correlate with increased KYN/TRP, suggesting increased IDO1 activity at diagnosis. Increased sIDO1 is linked to a pro-inflammatory status in CML patients, as it positively correlates with increased serum neopterin levels as well as to various other pro-inflammatory markers, such as IFN-γ, IL-10, IL-17A, sVEGF, sVEGFA, sP-selectin and sPAI-1, representing a highly mutated gene, IDO1 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. MR^4.5) 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 kynurenic acid in 10% of patients treated with ponatinib. In contrast, in CML an increased KYN/TRP ratio is associated with improved molecular response. Therefore, to implement testing of the endpoint, the IDO activity may reflect endogenous IFN-α production, a known factor favoring immune-mediated CML control. The predictive potential of KYN/TRP is currently verified in an independent cohort.

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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 ponatinib occurs in a considerable proportion of patients. The best-characterized mechanism of ponatinib 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 IC^50 values for this TKI exceed the maximum achievable effective plasma levels (eC^50). These co-called compound mutations (CMs) are associated with increased oncogenic potential in comparison to individual mutations, and represent a powerful mechanism of potential resistance to all currently available TKIs. The occurrence of compound mutations has been linked particularly to sequential treatment with different TKIs, and the identification of their responsiveness to ponatinib is of paramount importance for the subsequent clinical management.

Aims: 1. To determine the spectrum of highly TKI-resistant CMs. 2. Measure the responses of BCR-ABL1 CMs to ponatinib

Methods: We have established a BCR-ABL1 protein model facilitating assessment of the presumptive impact of 27 different CMs involving important functional sites of the BCR-ABL1 TKD, and including constellations expected to display 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.

Results: Most CMs involving sites with no previous evidence for implication in resistance to ponatinib displayed IC^50 values below 10 nM. This eC^50 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 IC^50 values, some of the predicted compound mutations containing F317L displayed an eC^50 for ponatinib in the range of the eC^50 achievable only with a daily dose of 45mg. Some of 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 advisable, therefore, to implement testing of the endpoint in drug concentrations and monitoring the kinetics of mutant subclones covering also compound mutations in the routine diagnostic surveillance to provide a basis for optimized clinical management of patients treated with ponatinib.

P597

IS THERE EFFECTIVE IMMUNE SURVEILLANCE AGAINST CHRONIC MYELOID LEUKAEMIA? NO

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Background: Immune surveillance refers to a process whereby the innate and adaptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (GVHD) and whether there is 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 attribute this therapy-free remission (TFR) to a result of immune surveillance. Immune surveillance refers to a process whereby the innate and adaptive immune systems recognize and eliminate cancer cells on the basis of cancer-specific or related antigens or other molecules. Substantial data support the concept of immune surveillance in rodents but data in humans are less convincing and apply mostly to virus-induced cancers. However, considerable data indicate a strong anti-leukaemia effect in persons with chronic myeloid leukaemia (CML) receiving an allogeneic haematopoietic cell transplant. Most, if not all, of this anti-leukaemia effect is confounded with graft-versus-host disease (GVHD) and whether there is a specific anti-leukaemia effect distinct from GVHD is controversial. 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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 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. The CTS dataset also included 1.72 (0.6, 4.0; P=0.34) representing 5 cases in 182,833 person-years at-risk vs. 3 expected (2 excess cases). Amongst heart transplant recipients the SIR was 3.47 (1.8, 6.1; P=0.0005) representing 12 cases in 173,015 person-years at risk vs. 3 expected (9 excess cases). Data from recipients of kidney and liver transplants suggest immune suppression does not increase risk of developing CML or does so very slightly. The increase in SIR in kidney graft recipients is generally attributed to increased cancer surveillance including blood testing. Although the SIR of CML was substantially-increased after heart transplants, these persons receive high doses of ionizing radiations for diagnosis and management procedures such as computer tomography (CT)-angiography. Ionizing radiations are a proved cause of CML which might explain the increased SIR. Results: Our data, 25 excess cases of CML in 2,038,339 person-years at-risk observation suggest the magnitude of immune-surveillance do not support the hypothesis that immune surveillance operates to an important extent to prevent CML in humans. Summary/Conclusions: Consequently, the anti-leukaemia effect associated with allotransplants and the TFR observed after stopping TKI-therapy is unlikely to result from effective immune surveillance against CML.

P599

MUTATIONAL ANALYSIS IN BCR-ABL1 POSITIVE LEUKEMIA BY DEEP SEQUENCING BASED ON NANOPORE MINION TECHNOLOGY

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Background: In newly-diagnosed chronic phase (CP)-CML patients, 15–30% who start first-line tyrosine kinase inhibitors (TKIs) therapy will not reach an optimal response, and a BCR-ABL1 kinase domain (KD) mutation will be detectable in 25–50% of patients with treatment failure with an increased frequency of these mutations observed in accelerated phase and blast crisis patients. Currently, Sanger sequencing (SS) technique analyzing BCR-ABL1 is considered the gold standard for mutation detection knowing that this assay 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, they are burdened by costs related to the initial investment, that is the sequencer purchase, the preparation of specific targets libraries, and the required reagents. MinION is a single molecule sequencer connected to a laptop through a USB3.0 interface, based on nanopore technology; it works by connecting two strands of DNA molecules by a hairpin, and sequencing them consecutively.

Aims: To describe a third-generation sequencing assay on MinION for detecting BCR-ABL1 KD mutations and compare the results to a SS-based test in 24 Ph+ leukemia cases.

Methods: Overall, 24 patients were included; among them, 12 (11 CML and 1 ALL cases) developed treatment resistance during the TKI’s treatment course (Group A). In total 10 and 4 cases (Group B) developing BCR-ABL1 kinase domain (KD) mutation will be detectable in 25–50% of patients with treatment failure with an increased frequency of these mutations were included in the present study. Data was obtained from peripheral blood cells. MinION library preparation, sequencing and data analysis were performed. All cases included in the study were analyzed by SS and MinION sequencing in blinded manner.

Results: Two sequencing runs were performed with the two different pools of patients: the first lasted eight hours and was carried on the Group 1, whereas 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, respectively. Any case detected as “positive” by MinION sequencing 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 depth 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 simulated around <20%) in 2 (22%) 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 mutation in Ph+ leukemias.

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THE AUTOMATED MOLECULAR TECHNIQUE “ULTRA” ALLOWS A SENSITIVE AND ACCURATE BCR-ABL1 QUANTIFICATION IN PATIENTS AFFECTED BY CHRONIC MYELOID LEUKEMIA

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Background: Chronic myeloid leukemia (CML) is characterized by the presence of thePhiladelphia chromosome and the BCR-ABL1 fusion gene. The production of tyrosine kinase inhibitors (TKIs) significantly improved the survival, but 15% of patients don’t reach the optimal responses at the defined end-points or develop secondary resistance. The 2013 ELN guidelines identified as fundamental the early molecular response (BCR-ABL1/ABL1 % ≤10% IS), the MR3 (<0.1%) and the deep molecular response (MR4<0.01%, MR4.5<0.005%, and MR5 <0.001%). Consequently, the molecular monitoring plays a crucial role in the clinical management of CML patients, with a consequent research of sensitive and standardized molecular techniques. The automated methods offer advantages in terms of reduced time for analysis, decreased manual steps, and reduction of possible errors and contamination.

Methods: Aims: First of all, 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 one. Methods: We assessed the BCR-ABL1 transcript in 86 patients afferent to laboratories of Pisa, Napoli, Torino, and Bologna (Italy). For statistical analysis, the χ2, the Pearson’s and the Cohen’s K test were adopted. Because our patients presented different transmogrification levels (from >10% to the 0%) (MR4, MR4.5, MR5) the two techniques have been compared in 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 early response (MR2+MR3). The automatic method (32.000 000 copies, 95% CI 24.000 000–40.000 000) vs. the manual method (27.000 000 copies, 95% CI 22.000 000–32.000 000). 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 with the LabNet method, the automated one: the percentage of discordance demonstrated high concordance between “Ultra” and “LabNet” methods using assay comparison criteria proposed by Müller et al. [Leukemia 2009] (Table 1).
and apoptosis in IM sensitive and resistant cell lines.

The advantage of using AK and Plk1 inhibitors in involved cell growth control and apoptosis.

- reduction of cell survival;

- associated with:

- activity. Subsequent experiments showed that the inhibition of AK A and Plk1 (T210). 24h exposure to IM significantly reduced expression and phosphorylation of AK A (S282) and Plk1 (T210). 24h exposure to IM significantly reduced expression and phosphorylation of AK A (S282) and Plk1 (T210). 24h exposure to IM significantly reduced expression and phosphorylation of AK A (S282) and Plk1 (T210).

- V/PI staining; cell cycle distribution was observed by PI staining and subsequent GADD45a interacts with Aurora Kinase A (AK A), a key component of centrosome cycle and polar spindle assembly required for regulated progression from G2 to G1 and throughout.

Background: Cell response to stress is a central component of genomic stability. The integrity of signaling pathways involved in cell cycle arrest, chromatin remodeling and DNA repair, are critical for the maintenance fidelity of replicated DNA. In this context, Gadd45 proteins function as stress sensors and regulate transcription regulators. Gadd45α, in particular, intervenes in G2/M checkpoint induction and DNA repair, and it is required for efficient coordination of centrosome duplication hence preventing aneuploidy and aneuploidy. Such evidences let assume a putative role of Gadd445α in cancer development and progression, supporting the idea that Gadd45α interacts with Aurora Kinase A (AK A), a key component of centrosome cycle and polar spindle assembly required for regulated progression from G2 to G1 and throughout.

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, s2y of frontline NIL, and MR4.5 (BCR-ABL1 ≤ 0.0032%) prior to enrollment. All pts provided informed consent. After enrollment, pts continued NIL for ≥2 y of frontline NIL, and MR4.5 during the consolidation phase; pts with no assessment worse than MR4 (8/20 (40.0% [95% CI, 19.1% - 63.9%]) were grouped according to Sokal risk score at diagnosis or depth of response and MR4.5 in the consolidation phase. 95% CI, 15.5% - 52.4% respectively, remained in TFR at wk 49 (Sokal risk scores were missing for 50 pts). Among pts with ≥2 assessments during the consolidation phase, 90.0% (95% CI, 45.2% - 60.6%) remained in TFR at wk 48 vs 82/40.0% (95% CI, 19.1% - 63.9%) who had ≥1 assessment between MR4 and MR4.5 during the consolidation phase. Overall, of 88 pts who reintegrated NIL due to loss of MMR, 87 (98.9%) regained MMR and the remaining pt left the study 7.1 wk after NIL reintiation 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 48 wk of TFR, 2.0% (1.0%) and 2.0% (1.0%) for pts with cardiovascular AEs during the first and second 48 wk of TFR, respectively; 34 (34.0%) and 9 (9.0%), respectively, had AEs in the predefined musculoskeletal pain group.

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 TFR. No strong predictive factors for remaining in TFR were identified. Pts with low Sokal risk and pts with continuous MR4.5 in the consolidation phase tended to have higher TFR rates than other pts, although these results must be interpreted with caution due to the small number of pts in some subsets and the wide 95% CIs. Additionally, the biological explanation for an association between Sokal risk score at diagnosis and a subsequent ability to remain in TFR is unknown. These results support TFR as a valuable option for pts in sustained DMR on frontline NIL.

Table 1.

P601

DURABLE TREATMENT-FREE REMISSION (TFR) FOLLOWING FRONTLINE NILOTINIB (NIL) IN PATIENTS (PTS) WITH CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): ENESTFREEDOM 96-WK UPDATE


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Background: ENESTfreedom (NCT01794068) is evaluating the ability to stop and maintain 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, s2y of frontline NIL, and MR4.5 (BCR-ABL1 ≤ 0.0032%) prior to enrollment. All pts provided informed consent. After enrollment, pts continued NIL for ≥2 y of frontline NIL, and MR4.5 during the consolidation phase; pts with no assessment worse than MR4 (8/20 (40.0% [95% CI, 19.1% - 63.9%]) were grouped according to Sokal risk score at diagnosis or depth of response and MR4.5 in the consolidation phase. 90.0% (95% CI, 45.2% - 60.6%) remained in TFR at wk 48 vs 82/40.0% (95% CI, 19.1% - 63.9%) who had ≥1 assessment between MR4 and MR4.5 during the consolidation phase. Overall, of 88 pts who reintegrated NIL due to loss of MMR, 87 (98.9%) regained MMR and the remaining pt left the study 7.1 wk after NIL reintiation 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 48 wk of TFR, 2.0% (1.0%) and 2.0% (1.0%) for pts with cardiovascular AEs during the first and second 48 wk of TFR, respectively; 34 (34.0%) and 9 (9.0%), respectively, had AEs in the predefined musculoskeletal pain group.

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 TFR. No strong predictive factors for remaining in TFR were identified. Pts with low Sokal risk and pts with continuous MR4.5 in the consolidation phase tended to have higher TFR rates than other pts, although these results must be interpreted with caution due to the small number of pts in some subsets and the wide 95% CIs. Additionally, the biological explanation for an association between Sokal risk score at diagnosis and a subsequent ability to remain in TFR is unknown. These results support TFR as a valuable option for pts in sustained DMR on frontline NIL.

P602

RESPONSE DIFFERENCES IN THE BCR-ABL1 E13A2 AND E14A2 VARIANTS MAY BE A TECHNICAL QC ARTIFACT

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Keywords: Chronic myeloid leukemia - Clinical 2
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 with the issue remaining controversial. Reverse transcription quantitative PCR (RT-qPCR) using the European Against Cancer (EAC) qPCR assay has been the gold standard for determining the levels of BCR-ABL1 transcripts. The assay use common primers for amplification of the two variants resulting in a PCR product for the e14a2 variant that is 75 base pairs longer than the e13a2 variant. Under suboptimal PCR conditions, amplicons may be amplified with different efficiencies, which can result in an underestimation of especially the amount of longer qPCR products.

Aims: To study the accuracy of the EAC assay in quantifying the e13a2 and e14a2 transcripts.

Methods: Patient samples were screened for BCR-ABL1 e13a2 and e14a2 transcript variants using either PCR with agarose gel separation or a droplet digital PCR (ddPCR) assay measuring the amount of e13a2 and e14a2 transcripts. The BCR-ABL1 level was determined by ddPCR using the QuantStudio instrument (Life Technologies) and expressed in the International Scale (%IS) using the EAC primers and assay conditions with GUSB and BCR as reference genes. Samples were re-measured by digital droplet PCR (ddPCR) on a QuantLife instrument (Bio-Rad) using modified EAC primers multiplexed with GUSB and BCR as reference genes and expressed as %IS.

Results: Transcript levels from 124 BCR-ABL1 positive patient samples were determined using the EAC qPCR assay (median: 0.08% IS, range: 0.001–159% IS) and ddPCR (median: 0.01% IS, range: 0.0002–124% IS). These included 59 samples with the e13a2 variant and 65 with the longer e14a2 variant. Comparing the expression levels obtained by the two techniques revealed ddPCR/qPCR ratio differences for e13a2 (median: 0.68, range: 0.35 - 3.2) and e14a2 (median: 3.43, range: 0–8.8), and a consistent 4.5 fold (>0.5 log) underestimation of the levels of the e14a2 compared to e13a2 when using qPCR (figure 1).

Summary/Conclusions: When we compared the BCR-ABL1 levels using qPCR and ddPCR, we observed a discrepancy between the e13a2 and e14a2 breakthrough variants. Since ddPCR is an endpoint measurement and not sensitive to variations in primer efficiencies, the most likely explanation for the discrepancy is a decreased ddPCR efficiency of the longer e14a2 variant compared to e13a2 variant. Thus in qPCR analyses using the EAC protocol this may, at least in some clinical platforms, result in a consistently underestimated 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.

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5-YR RESULTS FROM THE PIVOTAL PHASE 2 PONATINIB PACE TRIAL: EFFICACY, SAFETY AND LANDMARK ANALYSIS IN HEAVILY PRETREATED PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CP-CML)


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Background: Ponatinib is an oral tyrosine kinase inhibitor (TKI) approved for pts with CP-CML or Philadelphia chromosome–positive acute lymphoblastic leukemia (Ph+ ALL) for whom no other TKI therapy is indicated, or for pts with T315I. The ponatinib PACE trial (NCT01207440) enrolled pts with CML or Ph+ ALL resistant/intolerant to dasatinib or nilotinib, or with T315I. Long-term results in these heavily pretreated pts provide value in informing treatment decisions.

Aims: To report 5-yr efficacy and safety, and the association of early landmark responses with survival at 5-yr landmark assessments as at 4 yrs past landmark, in heavily pretreated pts with CP-CML from PACE.

Methods: Ponatinib starting dose was 45mg/day. Dose reductions were instructed in Oct ’13 to manage risk of arterial occlusive events (AOEs) observed with longer follow-up. Outcome measures were: 5-yr efficacy (n=267) and safety (n=270); post-study final analysis (n=267) of the association of molecular responses (BCR-ABL1 ≤0.1% [major molecular response (MMR)], >0.1–1%, >1–10% and >10%) and cytogenetic responses (major [MCyR] and complete [CCyR]) at 3-, 6- and 12-mos with progression-free survival (PFS) and overall survival (OS) 4 yrs past landmark (log-rank P values). Data cutoff: 3 Oct ’16.

Table 1.

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Results: Baseline characteristics of the CP-CML pts included: median time from diagnosis, 7 yrs (range, 0.5–27 yrs); median age, 60 yrs (18–94 yrs); median %Ph+ 100% (2.5–100%); median %Ph+ 20% (7%). 160 of 182 CP-CML pts received ≥3 prior TKIs. At initiation of study closure, 99 pts (37%) were on ≥3 prior TKIs. At initiation of study closure, 99 pts (37%) were on ≥3 prior TKIs. Among these pts, minimum follow-up was 52 mos, and most (78%) had 15mg/d as their last ponatinib dose. In efficacy-evaluable CP-CML pts, cumulative response rates as of the data cutoff were: MCyR, 60%; COyR, 54%; MMR, 40%; and MR4+, 24%. Among pts who achieved MCyR (n=148) or MMR (n=108), the Kaplan-Keier (KM) estimated probability of remaining in response at 5 yrs was 74% (95% CI, 62–83) and 61% (95% CI, 51–70), respectively. Maintenance of response was high regardless of dose reductions in Oct ’13. KM estimated 5-yr rates for PFS/OS were 49%/77%. Among pts with 3-, 6- and 12-mo landmark assessment, MCyR/CCyR rates were: 57%/45%, 62%/52% and 71%/56%, respectively, and MMR achieved in 14%, 29% and 39%, respectively. Achievement of cytogenetic response and deep reductions in BCR-ABL1 levels (Table 1) at most landmark time points was associated with improved PFS and OS 4 yrs past landmark. Deeper responses at all landmarks were associated with achievement of MMR. Overall, the incidence of adverse events (AEs) in ≥45% of CP-CML pts was rash 47%, abdominal pain 46%, and thrombocytopenia 46%. Most newly occurring AEs were observed within the first yr. The incidence of any AOE/serious AOE for CP-CML pts was...
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, clinical responses. Among CP-CML pts with no prior AOE who had a prospective dose reduction, 17% (11/63) had a first AOE occurring after Oct ’13. Among these, 208 (79% of all 263 patients) achieved a cytogenetic response (CyR), which was partial in 18 patients and complete in 52 patients (1% CyR). Among the 190 patients in CCyR, 148 (56.2%) achieved a molecular response (MMR) (ratio < 0.1%) after a median period time of 13.7 months (IQR 9.0-22.3). Eleven patients (4.2%) developed a blast crisis, with a median period for blast crisis of 3 cases. After a median follow-up of 45.0 months from IM start (IQR 22.3–72.0), 93 patients have died (9 from disease progression and 84 from unrelated causes), 144 are alive and 104 of them are still in treatment with IM, while 8 discontinued for prolonged deep molecular response and 22 switched to 2nd line TKI. Five-year event-free survival (EFS) and overall survival (OS) were 51.2% (CI95% 44.8-57.6) and 70.9% (CI95% 64.6-77.2), respectively. At univariate analysis Hb level at diagnosis (≥ 12 vs < 12g/dl, p=0.021) and the initial dose of IM (400 vs ≤300, p=0.048) were significant predictive factors for OS, while PLT count at diagnosis (< 500 vs ≥ 500, p=0.006) and female gender (p=0.046) were significant predictive factors for MMR achievement. Multivariate analysis for EFS and OS are described in Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Multiivariate analysis for EFS</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 75</td>
<td>1.39</td>
<td>1.04-1.60</td>
<td>0.02</td>
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<tr>
<td>Male gender</td>
<td>1.29</td>
<td>1.10-1.51</td>
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<td>Disease status</td>
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<td>0.98-1.10</td>
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<tr>
<td>Multifocal analyzation for OS</td>
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<td>0.89-1.08</td>
<td>0.03</td>
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<tr>
<td>Male gender</td>
<td>1.29</td>
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<td>0.89-1.08</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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.
ease (including 7 acute myocardial infarction), 8 PAOD, 4 carotid stenosis (asymptomatic), 2 avascular necrosis of femoral head, 1 optic artery ischemia, 1 aneurysm of aorta/right iliac artery. Overall, 21 patients were hospitalized for the management of ATEs; 15 patients received medical treatment only, while the remaining required invasive interventions: 9 coronary angioplasty with stent positioning, 3 lower limbs amputations, 2 peripheral vascular bypasses, and 1 prosthesis of femoral head. No patient died for ATEs. Overall, 24 patients (80% of patients with ATEs, and 7% of the whole cohort) permanently discontinued nilotinib because of ATEs. The median follow-up after ATE was 15 (1-58) months. Of the 30 patients with ATEs, 26 (87%) achieved a MMR and 18 (60%) obtained a MR4, during nilotinib treatment. These rates were comparable to those observed in patients without ATEs (MR3: 260/315, 83%; MR4: 113/315, 64%). The 5-year progression-free survival and overall survival rates 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.

P606

ASSESSMENT OF CHRONIC RENAL INJURY IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN THE CHRONIC PHASE RECEIVING TYROSINE KINASE INHIBITORS

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Background: Long-term use of tyrosine kinase inhibitors (TKIs) may lead to chronic renal injury.

Aims: To evaluate the incidence of chronic kidney disease (CKD) in patients with chronic myeloid leukemia (CML) in the chronic phase (CP) receiving TKIs, and to identify the factors associated with the onset of CKD.

Methods: Data of CML-CP patients treated with TKIs as first-line or second- or third-line therapy for at least 3 months were analyzed. Glomerular filtration rate (GFR) was followed from the initiation of TKI therapy. CKD was defined as persistent GFR less than 60 ml/min/1.73 m2 or persistent more than 30% GFR reduction from baseline. CKD-free survival was used to evaluate the onset of CKD. Patients’ characteristics and TKI used were analyzed to identify the factors associated with the onset of CKD by Cox regression model in those receiving first-line and second- or third-line TKI therapy, respectively.

Results: 587 patients were included in this study. 383 (65%) were male. Median age was 40 (17-84) years. 464 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 imatinib use (HR=2.4, 95% CI 1.4-4.3, p=0.002), male gender (HR=2.0, 95% CI 1.4-2.9, p=0.001), increasing age (HR=1.2, 95% CI 1.1-1.4, p=0.003) and prior administration of hydroxyurea, interferon or chemotherapy (HR=1.7, 95% CI 1.1-2.8, p=0.010) were factors associated with incident of CKD. In 48 patients with abnormal GFR or prior CKD before first-line TKI-therapy, 8 of 42 (19%) developed ≥30% GFR reduction from baseline during nilotinib or dasatinib-therapy. In 123 patients receiving nilotinib (n=59) or dasatinib (n=64) as second- or third-line TKI-therapy after imatinib-failure, 13 of 110 (12%) with normal GFR at baseline developed CKD with a median follow-up of 19 months (range, 3-149 months). Probabilities of CKD-free survival at 3 years were 74% and 90% in those receiving nilotinib and dasatinib, respectively (p=0.059). Multivariate analysis showed that male gender (HR=1.8, 95% CI 1.1-3.0, p=0.023) and a history of diabetes mellitus, hypertension or other renal diseases (HR=3.8, 95% CI 1.3-11.6, p=0.019) were factors associated with incident of CKD. 3 of 13 (23%) patients with abnormal GFR or prior CKD before second- or third-line TKI-therapy developed ≥30% GFR reduction from baseline during nilotinib (n=1) or dasatinib (n=2) therapy.

Summary/Conclusions: Our study showed that nilotinib and dasatinib were associated with less chronic renal injury compared with imatinib as first-line TKI-therapy, while dasatinib was related to less loss of renal function compared with nilotinib as second- or third-line TKI-therapy after imatinib-failure in CML-CP patients.

P607

COMPARATIVE MONITORING OF MINIMAL RESIDUAL DISEASE (MRD) BY qPCR AND DIGITAL-PCR (dPCR) IN CHRONIC MYELOID LEUKEMIA PATIENTS ACHIEVING MAJOR OR DEEP MOLECULAR RESPONSE WITH TIIROSIN-KINASE INHIBITORS

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Background: Quantification of BCR-ABL1 transcript by qPCR is mandatory to monitor the response to TKIs therapy in CML patients. The achievement of Major or Deep Molecular Response (MMR or DMR) with TKIs is crucial for long-term survival and for treatment free remission (TFR). Currently, up to 30-40% of CML patients treated with TKIs can achieve DMR, but 50-60% of deep responders who discontinue the treatment loose their DMR and re-challenge into continuous TKIs therapy. qPCR has some intrinsic limitations and it does not appear to be an optimal assay to select the best candidates to TKIs discontinuation. Digital PCR (dPCR) can give an absolute quantification of target nucleic acids by partitioning the PCR reaction mix over a large number of wells, each containing a single copy or no copies of the target region.

Aims: The aim was to comparatively monitor the BCR-ABL1 transcript levels by dPCR and qPCR in 57 CML patients treated with TKIs and achieving MMR or DMR in at least 3 time points.

Methods: Using qPCR and dPCR (Q530 Digital PCR System by Life Technologies), we comparatively analyzed 228 peripheral blood samples from 57 CML patients with MMR (n=14) or DMR (n=43). qPCR analysis were performed according to the last International Guidelines while absolute quantification of BCR-ABL1 transcript were obtained by dPCR and results were expressed as number of BCR-ABL1 copies/ul of reaction. Patients were divided into 3 groups corresponding to the MR classes at the first time point: MR3.0, MR4.0 and MR4.5-5.0 groups. dPCR Positive Predictive Value (PPV) was also preliminary evaluated in 14 patients undergoing TKI discontinuation.

Figure 1. Results: Analyzing comparatively the time course of MR in the patients of the three groups (MR3.0, MR4.0 and MR4.5-5.0) it was observed a similar trend, but the dPCR allowed to appreciate that, at the time of starting the monitoring the patients showed different levels of BCR-ABL1 copies/ml. Furthermore, those patients with MR4.5-5.0 undetectable by qPCR resulted with detectable BCR-ABL1 transcript levels when assessed by dPCR. Secondy, while MRD quantitations measured by qPCR appear to be more homogeneous, nearly due to a normalization effect of qPCR, the quantitations of MRD measured by dPCR appear to be more heterogeneous because of the high sensitivity and accuracy of dPCR. Therefore, dPCR values, reflecting the great heterogeneity of MRD level in patients belonging to the same MR group, suggest a higher accuracy in patients stratification (Figure 1a). dPCR value of 0.468 copies/ul, previously reported as value discriminating between major responders and deep responders, was used as threshold for dPCR data analysis. Patients with absolute value of BCR-ABL1 lower than 0.468copies/ul at the first time point presented more stable disease levels than the patients with absolute value of BCR-ABL1 higher than 0.468copies/ul (Figure 1b). In 14 CML patients who
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: The overall survival (OS) of our cohort was 31.87 months (mo). For patients with progression to BP-CML from previously known diagnosis of CML, median time to progression was 19.1 mo (range: 3.0-221.2 mo). The median OS from the diagnosis of BP-CML in this cohort was 10.8 mo, compared to de novo CML-BP cohort OS of 11.03 mo (p-value=0.62). Myeloid blast phase CML had worse OS compared to lymphoid blast phase cohort but was not statistically significant (9.17 vs 17.5 mo, p=0.32). We further compared the treatment strategies of BP-CML including single agent TKI (n=21) and conventional chemotherapy regimens in combination with a TKI (n=36). The median OS of the cohort with single agent TKI was not statistically different from the combination with chemotherapy arm (12.83 mo vs 10.87 mo, p=0.73) as shown in Figure 1A. Additionally, combination of chemotherapy with TKI compared to single agent TKI did not have significant survival impact in either myeloid (9.17 vs 9.13 months, p=0.32) or lymphoid (14.47 vs 18.27 mo, p=0.24) BP-CML. Total of 26 patients (44.8%) proceeded to allogeneic bone marrow transplant, 26% (n=6) of which only received TKI prior to transplant compared to 76.9% (n=20) who received chemotherapy in combination with TKI. Use of single agent TKI rather than TKI in combination with chemotherapy prior to allogeneic transplant had a trend toward improved OS (128.5 vs 24 mo, p=0.23) (Fig 1B). Choice of TKI in combination with chemotherapy in treatment of BP-CML also did not identify any TKI combination resulting in superior survival (Figure 1D). Overall survival of the cohort stratified by presence of standard Philadelphia chromosome in comparison to additional cytogenetic aberrations did not detect difference in overall survival (10.87 vs 12.1 mo, p=0.51). Further evaluation of cytogenetic aberrations revealed monosomy 7 to be present in greater frequency in lymphoid blast phase compared to myeloid blast phase (35.7% vs 6.29%, p=0.02).

Summary/Conclusions: Our data suggest no survival difference when BP-CML is treated with a single agent TKI compared to a combination therapy, regardless of histology type. Therefore, single agent TKIs should be considered as an effective frontline therapy option for BP-CML, which may prevent the potential toxicity associated with chemotherapy. These findings need further validation in a larger prospective cohort.

P609
EFFICACY OF SWITCHING TO DASATINIB IN CHRONIC MYELOID PATIENTS WITH LATE WARNING RESPONSES TO IMATINIB. STUDY OF THE ASSOCIATION OF RESPONSE TO DASATINIB TO IMMUNOLOGIC STATUS
J.L. Steegmann1,*, V. Garcia-Gutiérrez2, B. Colom3, F. Sanchez-Guijo4, R. Ayala5, C. Boque5, F. Casado5, B. Xicoy5,1, M. Montero5, C. Soto5,10, R. de Paz5,1, A. Kreuzman5, J. Martinez-Lopez2, C. Munito2
1Servicio de Hematologia, Hospital Universitario de la Princesa/ IIS-IP Madrid, 2Servicio de Hematologia, Hospital Universitario Ramón y Cajal, 3Servicio de Immunologia, Hospital Universitario de la Princesa, Madrid, 4Servicio de Hematologia, Hospital Universitario de Salamanca, Salamanca, 5Servicio de Hematologia, Hospital Duran i Reynals, Barcelona, 6Servicio de Hematologia, Hospital Virgen de la Salud, Toledo, 7Servicio de Hematologia, Hospital Germans Trias i Pujol, Barcelona, 8Servicio de Hematologia, Hospital Universitario Virgen del Rocio, Sevilla, 9Servicio de Hematologia, Hospital Povisa, Vigo, 10Servicio de Hematologia, Hospital Universitario La Paz, Madrid, Spain

Background: European LeukemiaNet (ELN) recommendations (2013) advised closely monitoring for patients with late warning response (patients with complete cytogenetic response without major molecular response after 12 months of treatment). Our trial, DASAPOST, has been the first one evaluating efficacy and safety of dasatinib in patients with late warning responses, and preliminary results have been reported (García-Gutiérrez et al, ASH 2016; P5450). Besides, many studies suggest that dasatinib may augment responses due to its immunomodulating effect. Although NK and CD8 cells seem to be involved, the specific mechanism remains to be clarified.

Aims: To evaluate the efficacy and safety of switching change to dasatinib in patients treated with imatinib first line during at least 18 months and having a late warning response, and to study the association between response to dasatinib and immune robustness, both baseline and during the therapy, and dasatinib-induced lymphocyte mobilization.

Methods: Phase II, open, multicenter DASAPOST study (NCT01802450). Patients previously treated with imatinib after at least 18 months, with CCyR but without MMR, were included. All BCR-ABL1/ABL (IS) measurements were centralized in a EUTOS laboratory. Analysis of HLA class I in some point were considered as non responders. Lymphocyte counts, subpopulations and migration studies were done at baseline (1st day of dasatinib), and every 3 months, and safety of dasatinib in patients with late warning responses, and preliminary results have been reported (García-Gutiérrez et al, ASH 2016; P5450). Besides, many studies suggest that dasatinib may augment responses due to its immunomodulating effect. Although NK and CD8 cells seem to be involved, the specific mechanism remains to be clarified.

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Table 1.

<table>
<thead>
<tr>
<th>Lymphocytes Baseline</th>
<th>CD3 Baseline</th>
<th>CD4 Baseline</th>
<th>CD8 Baseline</th>
<th>NK Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (× 10⁹/L)</td>
<td>1.78 (0.82-2.24)</td>
<td>1.40 (0.92-1.68)</td>
<td>0.90 (0.41-1.29)</td>
<td>0.20 (0.09-0.87)</td>
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<td>Percentage</td>
<td>27.2 (14.5-19.8)</td>
<td>29.2 (23.0-36.8)</td>
<td>51.4 (46.4-56.3)</td>
<td>14.0 (11.6-16.3)</td>
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Table 2.

<table>
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<th>Lymphocytes Baseline (× 10⁹/L)</th>
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<tbody>
<tr>
<td>MMPM</td>
<td>2.23</td>
<td>1.63</td>
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<td>Lymphocytes Baseline (× 10⁹/L)</td>
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<td>CD3 Baseline (× 10⁹/L)</td>
<td>0.62</td>
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<tr>
<td>CD8 Baseline (× 10⁹/L)</td>
<td>0.67</td>
<td>0.29</td>
<td>0.084</td>
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</table>
Summary/Conclusions: Our study shows that in patients treated with imatinib and with late warning responses, switching to Dasatinib induced MMR in 2 out every 3 patients, and MR4 in half of the patients, with a good safety profile. Contrarily to other group reports, we have not found any significant association between response and lymphocyte mobilization in any point studied. Interestingly, the absolute number of CD8 at baseline was significantly associated with the early 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.

Methods: The BCR-ABL measurements acquired using EAC and in-house modified EAC protocol have been compared with results from SA Pathology in Adelaide. The Adelaide protocol (Branford and Hughes 2006) consists of separate, optimized reactions for e13a2 and 14a2 transcripts, therefore it should be considered free of any PCR efficiency-related artifacts. The data originated from four independent sample batches exchanged between Poznan and Adelaide since 2009.

Results: The analysis of retrospective EAC protocol data showed that when e13a2 and e14a2 samples entered the exponential phase at the same time, the latter would cross the threshold approximately 2.2 cycles after the first one. Re-analysis of data from sample exchanges from 2009 revealed that after establishing a conversion factor (CF), all of the e14a2 measurements in Poznan were underestimated according to Adelaide. At the same time, almost all of e13a2 samples were overestimated (fig. 1). Still, the bias between methods was acceptable and a valid conversion factor (CF) was calculated. The method modification introduced 2011 eliminated this difference and increased concordance between laboratories. The last sample batch revealed significant difference between non-modified and modified EAC protocols in e13a2 measurements: 4.56 (+/- 0.96). Reanalysis of sample batch from 2009 (presented on fig.1) using 4.57 (2x2.28) factor (e13a2 results divided by 2.28, e14a2 results multiplied by 2.28) resulted in almost perfect data alignment. The results of modified EAC protocol, after CF recalculation, showed very good concordance with Adelaide (100% results of e14a2 and 88% of e13a2 within 2-fold of reference laboratory).

Methods: The genealogy 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, ANKRD55/MAP3K1, PDGFC, LYPLAL1, RSPO3, and FAM13A1 genes. The uGRS was based on the sum of the risk alleles within the set of selected SNPs.

Results: MR2.5 and CMR were achieved in 91% and 84% of the patients, respectively. Before treatment, none of the patients had abnormal blood glucose. During treatment and subsequently follow-up of 84.4 months (range 1-298), 5 patients (11%) developed diabetes requiring oral treatment, after a median of 11 months (range 3-95) since nilotinib. Nine patients (20%) developed prediabetes. Prediabetes/diabetes-free survival was significantly higher in patients with an uGRS below 10 compared to higher scores (100% vs 18%, p=0.004) (Figure). Each increment of 1 unit on the uGRS caused a 42% increase in the prediabetes/diabetes risk (HR=1.42; CI: 1.04-1.94; p=0.026).

Figure 1.

Summary/Conclusions: 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 an inherited 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 erythroid precursor (HDEP) 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. 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 Gene_cards (http://genecards2.dacya.ucm.es/)

Results: We first generated a founder female mouse lacking the intron 1 enhancer region of Alas2, including the GATA binding domain (Alas2^{delin1X}). Whereas the heterozygous Alas2^{delin1X/+} mice were viable and did not show any anemic phenotype, hemizygous deletion (Alas2^{delin1X/-}) 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 the 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 changes in the expression of GATA-1, MDS2, and ALAS2 as well as globin genes (HBA, HBB, and HBD) 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, HBB, HBM, and HBG) as well as genes involved in iron/heme metabolism (ALAS2, transferrin receptor: TFRC, coproporphyrinogen 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 cell osmolality, at least 50% reduction of IgM and improvement of any circulatory symptoms.

Conclusion: Bendamustine and rituximab combination therapy related to 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.

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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, in some cases, cold-induced circulatory symptoms. Pharmacological therapy, although not indicated in patients with very mild disease, seems required in a majority of cases. Corticosteroids are ineffective. Rituximab monotherapy has resulted in approximately 50% response rate and 1-year median response duration. Fludarabine and rituximab combination therapy showed 70% response rate (20% complete responses) and very long response duration, but considerably toxic.

Aims: We wanted to investigate whether bendamustine and rituximab combination therapy can result in favorable response rates and duration with an acceptable toxicity profile.

Methods: We conducted a prospective, uncontrolled multicenter trial with 16 participating hospitals from Norway, Finland and Denmark. Essential inclusion criteria were verified CAD with symptomatic anemia and/or severe cold-induced circulatory symptoms. Eligible patients received 4 cycles of rituximab 375mg/m² day 1 and bendamustine 90mg/m² day 1-2 with 28 days interval. Outcomes were assessed by clinical panel into complete response (CR), partial response (PR), and non-response (NR). The definition of CR included normalization of hemoglobin (Hb) levels with no hemolysis, complete histologic resolution of the bone marrow LPD and disappearance of monoclonal serum protein. The criteria for PR included increase in Hb levels by at least 2.0 g/dL or to the normal range, transfusion independence, at least 50% reduction of IgM and improvement of any circulatory symptoms.

Results: Forty-four patients (19 men and 25 women) were included, with a median age of 74 years (range, 48-86) and median disease duration 4 years (range, 0-18). Seventeen patients had received previous therapy. At baseline, median Hb level was 9.5g/dL (range, 4.5-14.8), bilirubin 65micromol/L, lactate dehydrogenase (LDH) 478U/L, haptoglobin undetectable, IgM 4.1g/L (1.0-2.72), CA litter 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 significantly higher of median of 4g/dL in the responders. 4.4g/dL in patients achieving CR and 3.9g/dL in those achieving PR. Median post-therapy Hb levels were 14.2g/dL (CR), 12.5g/dL (PR) and 10.5g/dL (NR). Acrocyanosis and Raynaud symptoms resolved completely in 16 patients and improved in 11 (47% and 32%, respectively, of those with such symptoms at baseline). Histologic regression of the LPD was complete in 17 patients (39%), partial in 5 (11%) and not evaluable in 18 (41%). Median time to response was 2 months (0.5-12). Only 3 responders experienced relapse; 2 after PR and 1 after CR. Median observed response duration was 32 months (range, 1-62) during median 32 months follow-up. Complete response, complete remission in 7 patients, very mild, and very mild disease, seems required in a majority of cases. Corticosteroids are ineffective. Rituximab monotherapy has resulted in approximately 50% response rate and 1-year median response duration. Fludarabine and rituximab combination therapy showed 70% response rate (20% complete responses) and very long response duration, but considerably toxic.

Conclusion: 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.

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EX VIVO TREATMENT OF RED BLOOD CELLS FROM 15 PYRUVATE KINASE (PK) DEFICIENT PATIENTS WITH AG-348, AN ALLOSTERIC ACTIVATOR OF PK-R, INCREASES ENZYMIC ACTIVITY, PROTEIN STABILITY AND ATP LEVELS

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Background: Pyruvate kinase (PK) deficiency is a rare hereditary disorder affecting red blood cell (RBC) glycolysis. It is caused by mutations in the PKLR gene. PK-deficient RBCs are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the upstream metabolite 2,3-diphosphoglycerate, and deficiency in the PK product ATP. It is hypothesized that insufficient energy production affects red cell homeostasis, promoting...
Methods: Aims: Generation sequencing (NGS) based panel that contained genes already to RBC membrane disorders.

Aims: To evaluate the effect of AG-348 treatment on PK-R enzymatic function, RBC metabolism and deformability

Methods: Observational case-control study, approved by the Institutional Review Board. All patients gave informed consent. Enrolled patients (N=15) were adults, transfusion-independent and compound heterozygous or homozygous for PKLR mutations. Baseline 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 Lorrcra) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 2 μM AG-348 (37°C) prior to test. Baseline protein levels of PK-R were assessed using antibodies against PK-R

Results: Baseline patient characteristics show strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Distinct metabolic changes were consistent with a block of glycolysis at 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 1.5-fold, range 1.2-3.4). Similar increases were observed in control cells (mean fold increase 2.3, range 1.2-7.1). ATP levels in PK-deficient cells increased upon AG-348 treatment (mean fold increase 1.2, range 0.2-2.2) similar to controls (mean fold increase 1.6, fold range 1.4-1.8). Generally, PK-R thermal stability was strongly reduced in PK-deficient patient cells, illustrated by a mean loss of activity of 72% (19% for control cells) after incubation at 53 °C for 60 minutes. Ex vivo treatment with AG-348 prior to incubation resulted in residual activity of 4 to >10-fold higher than residual activity of vehicle-treated samples. Baseline protein level analyses suggests that a certain level of PK-R protein is required for cells to respond to AG-348 treatment ex-vivo, as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R. In approximately half of the patients, ex vivo treatment with AG-348 was associated with an increase in RBC deformability, although there doesn't appear to be a clear correlation with enzymatic or metabolic response.

Summary/Conclusions: These data support the hypothesis that drug intervention with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of PKLR genotypes. The combination of PK-R enzymatic activity and ATP levels suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attracive way to correct the underlying pathologies of PK deficiency

Table 1. Baseline characteristics and genotypes of PK-deficient patients

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<th>Patient</th>
<th>PKLR mutation</th>
<th>Hb (g/dl)</th>
<th>LDH (IU/L)</th>
<th>ALT (IU/L)</th>
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<th>RBC (x10^6)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>Ret (%)</th>
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P615 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) adoption. Herein, we report on the genetic analysis of an NGS panel suggested by Sanger by several membrane related genes, considering that they all contain a high number of coding regions.

Aims: The aim of this study is to perform the molecular diagnosis of the patients included in the study as well as to identify new pathogenic mutations leading to RBC membrane disorders.

Methods: 116 patients from 74 unrelated families were studied with a next generation sequencing (NGS) based panel that contained genes already described as disease causing for RBC membrane disorders (ANK1, EPB41, EPB42, SLCA4A1, SPTA1, SPTB, PIEZO1, KCNN4, RHAG) as well as for enzymopathies (ADA, AK1, ALDOA, BPOM, CYB5A, G6PD, GCLC, GPI, GSR, GSS, HK1, NTS3CA, FFMK, PKG1, PKL1, TP11), hemoglobinopathies (HBA1, HBA2, HBB) and congenital disserthritoid anaemias (CDAN1, C15orf41, SEC23B, KLF1, GATA1, KIF23). The patients analysed were oriented as hereditary spherocytosis (63 patients), hereditary elliptocytosis or piroplasmocytosis (19 patients) and hereditary xerocytosis (8 patients). There were also 42 patients whose the combination of phenotypic laboratory results was suggestive of membranopathy 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. 48% (36/74) of the identified variants were missense changes, mostly from SPTB gene (11 variants), while a 38% (28/74) of the variants were nonsense changes and other type of variation such as frameshift mutation, mostly from ANK1 (12 variants) and SPTB (9 variants). Of special interest, only 2 variants were identified in more than one unrelated family: 1) SPTB c.647T>G, leading to splicingiscytosis, was identified in 8 patients of 2 unrelated families, 2) SPTA1 c.460G>1462dupTG, leading to elliptocytosis, was identified in 6 patients from 5 different unrelated families.

Finally, with the NGS panel results, the genetic diagnosis of 89% (103/116) of the included patients could be determined and only in 11% (13/116) the mutation was not identified or the variant correlation with disease was not clear. It is worthy to highlight that 10 of the 13 undiagnosed patients had been oriented as unclear membranopathy.

P616 CLINICAL FOLLOW-UP OF 378 PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA: PROGNOSTIC IMPACT OF HEMOGLOBIN LEVELS, AUTOANTIBODY CLASS, AND RETICULOCYTOPENIA AT ONSET ON THE RELAPSE RISK AND OUTCOME

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Background: Autoimmune hemolytic anemia (AIHA) is generally 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 10 sites, followed-up for 4.3 yrs (range 0.5-27). Patients 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 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),
Results: Table 1 shows clinical and laboratory characteristics of AIHA cases at onset and distribution of thermal types. HB values were significantly lower in IgG+C wAIHA and atypical cases (p<0.001), LDH higher in IgG+C wAIHA, mixed and atypical forms (p=0.01), and HB and LDH values were negatively correlated (r=-0.25, p=0.001). Absolute reticulocytes were reduced in CAD, mixed and IgG+C wAIHA (p<0.001) together with inadequate reticulocytosis (p=0.01). Moreover, the reticulocyte index was lower in cases with HB<6 g/dL (65 vs 98, p=0.01), along with more frequent inadequate reticulocytosis (87 vs 70%, p=0.01). First line therapy was administered in almost all cases but 25 CAD. A second therapy line was mostly required in IgG+C wAIHA, mixed, and to a lesser extent in CAD (p=0.005). The ultra-refractory cases requiring 4 or more lines of therapy were mainly mixed, atypical and CAD. Considering anemia severity, patients with HB<8 g/dL, more frequently required treatment after first-line (51 vs 33%, p=0.004; p=0.03), or even 3 or more therapy lines (52/71, 73% vs 19/71, 26%, p<0.001). The following hazard ratios (HR) emerged from multivariate Cox regression analysis: HR 3.2 (95% CI 1.4-7.9) 2.9 (1.4-6.2) 3.4 (0.4-21.5) 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).

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HEMINE BINDS ANNEXIN-A5 DURING HEMOLYSIS AND PREVENTS ITS INTERACTION WITH CELL MEMBRANE PHOSPHATIDYLSERINE DURING SICKLE CELL DISEASE

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Background: Intravascular hemolysis, such as in sickle cell disease (SCD), is characterized by extensive red blood cell damage, 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 extravascularization at the surface of cells and MP. Annexin-A5 is thought to orchestrate vesicle trafficking, promote cell membrane repair, protect against PS-mediated effects and enforce anti-inflammatory and anti-thrombotic control.

Aims: We investigated a possible functional relationship between intravascular hemolysis and annexins. We hypothesized that annexins, and annexin-A5 activity in particular, is blocked by intracellular heme as it is released in plasma during intravascular hemolysis.

Methods: In order to test the heme-annexin-A5 relationship, we measured PS-annexin-A5 binding of exogenous annexin-A5 to purified PS+ MP and plasma MP, as well as their subsequent detection by flow cytometry. Together, our data suggest that PS-neutralizing 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.

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USE OF PEGYLATED-CARBONYLHEMOGLOBIN BOVINE FOR THE TREATMENT OF SICKLE CELL DISEASE ASSOCIATED LEG ULCERS: RESULTS FROM A PHASE 2 SAFETY STUDY

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Background: Leg ulcers are a common complication of sickle cell disease (SCD). The pathophysiology of SCD leg ulcer is complex and may include obstruction of blood vessels by sickled red cell, chronic anemia, depleted nitric oxide bioavailability (resulting in impaired endothelial function), infection, thrombosis and excessive vasoconstriction. These events lead to progressive peripheral vasoconstriction and tissue necrosis, such that even minor lower-leg wounds can become persistent ulcers, with no tendency to heal after months of appropriate treatment. PEGylated-Carbonylhemoglobin bovine (PEG-COHb; SANGUINATE) is an oxygen carrying agent with anti-inflammatory activity. A study of safety and effectiveness was undertaken in SCD patients with chronic leg ulcers to determine the safety of this investigational drug administered in as a once weekly infusion for either 4 or 6 weeks.

Aims: To assess the safety and efficacy of repeated doses of PEG-COHb on SCD leg ulcers.

Methods: The study was an escalating, repeated-dose, open-label, Phase 2 study to test PEG-COHb at 320mg/kg (8 mL) in subjects suffering from leg ulceration associated with SCD. It was conducted in Panama and the Dominican Republic. All enrolled subjects underwent a 3-week Run-in Period, during which they received standard of care treatment for wound management. During the Treatment Period, subjects were assigned sequentially to Cohort 1 or Cohort 2. Cohort 1 received 4 once-weekly doses by 2-hour intravenous infusion of SANGUINATE. Following the completion of Cohort 1, the safety findings were reviewed prior to initiating Cohort 2. Cohort 2 received 6 once-weekly infusions. In addition to the study drug, subjects continued to receive standard of care during the Treatment Period. One week after the end of Treatment, subjects returned to the study center for a Final Visit. The following assessments were done: safety: Safety was assessed by recorded adverse events (AEs), laboratory tests (hematology, chemistry, and urinalysis), vital signs, concomitant medications, and 12-lead electrocardiograms (ECCs), efficacy: wound pain, wound appearance and condition, wound size, wound vascular status (Digital Vascular Severity Score (DVSS)), quality of life was assessed using the Short Form-12 v2 Health Survey (SF-12).

Results: The administration of once-weekly infusions of PEG-COHb was well tolerated. Treatment emergent adverse events (mild pyrexia, moderate wors-
ening anemia) considered related to study drug were reported in 2/10 patients. Increases in mean hematocrit 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 ulcers or 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.

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NON-RENAI N DETERMINANTS OF ENDOGENOUS ERYTHROPOIETIN LEVELS IN SICKLE CELL DISEASE
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Background: Sickle cell disease (SCD) is characterized by chronic hemolysis and inflammation. Elevated levels of erythropoietin (EPO) drive expansion of erythropoiesis to compensate for increased red cell destruction. EPO is produced in response to anemia and tissue hypoxia. Previous studies in SCD suggest that EPO is inappropriately low for the degree of anemia but the reasons are unclear.

Aims: To perform a retrospective analysis of data collected as part of routine clinical care to examine the relationship between serum EPO and degree of anemia, inflammation, hemolytic rate, alpha globin status, hemoglobin, alpha globin status, inflammation, serum ferritin and renal function.

Methods: King’s College Hospital (London, UK) has a large SCD population. All patients with HbSS or HbSBthalassemia who had a serum EPO level measured between 2007 and 2013 were included. Sickle genotype, alpha globin genotype, alpha globin status, ‘baseline’ HbF (no hydroxy carbamidone, transfusion or pregnancy) and demographic data were recorded. Other clinical variables were obtained from the same day as EPO levels (medications, laboratory values and oxygen saturation). Serum EPO was measured by chemiluminescence immunoassay (Siemens Immulite XPi). Exclusion criteria were: active vaso-occlusive crisis, transfusion within 8 weeks, chelation, erythropoiesis stimulating agent therapy, and inflammatory markers >30). Data analysis was performed in IBM SPSS Statistics 22. Skewed variables were log transformed and estimated QFR was calculated using the MDRD formula. Normalized variables were correlated with Ln EPO using Pearson correlation, ordinal variables using one way ANOVA, and binary variables using independent samples 2-tailed t-test. Multivariate linear regression using Ln EPO as the outcome was performed.

Results: 245 adult patients (12–17 years) SCD patients (all of African or African-Caribbean origin) met the inclusion criteria. Of the 245, 241 had HbSS and 4 HbSβthalassemia. Participants were enrolled after obtaining written informed consent/assent. This study is being conducted in 2 parts: Part A, single-dose, and Part B, multiple-dose for 24 weeks. The primary objective of Part A is PK and the primary objectives of Part B are safety and efficacy. PK samples to measure whole blood and plasma GBT440 concentrations were collected up to 15 days following single dose administration. Separate PPK models were developed to describe the concentration versus time profiles of GBT440 in whole blood and plasma using non-linear mixed effects modeling (NONMEM, version 7.3). The allometrically scaled adolescent PK model was also used to estimate the appropriate single dose for subsequence evaluation in pediatric participants (6 to 12 years).

Results: Part A has been completed; 7 adolescents (3 males/4 females) received a single dose of GBT440 600mg. The median age of participants was 16 years (range 14 to 16 years) and the mean weight was 52.8 kg (range 44.6 to 65.8kg). GBT440 was well tolerated; there were no drug-related ≥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.

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 PK model can potentially be used to estimate individual PK parameters (e.g., AUC) to support future GBT440 dose selection for evaluation in the pediatric population.

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 HbSBthalassemia). 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 PK model was also used to estimate the appropriate single dose for subsequent evaluation in pediatric participants (6 to 12 years).

Results: Part A has been completed; 7 adolescents (3 males/4 females) received a single dose of GBT440 600mg. The median age of participants was 16 years (range 14 to 16 years) and the mean weight was 52.8 kg (range 44.6 to 65.8kg). GBT440 was well tolerated; there were no drug-related ≥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.

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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 PK model can potentially be used to estimate individual PK parameters (e.g., AUC) to support future GBT440 dose selection for evaluation in the pediatric population.
Gene therapy, cellular immunotherapy and vaccination

P621

DEVELOPMENT OF TAX-REDIRECTED T-CELL IMMUNOTHERAPY FOR ADULT T CELL LEUKEMIA
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Background: Adult T cell leukemia/lymphoma (ATL) is an aggressive peripheral T-cell neoplasm caused by HTLV-1 virus infection and its prognosis remains very poor. Tax, which is the most important regulatory protein of HTLV-1, is associated with aggressive 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 autologous stem cell transplantation (allo-SCT). We found that a particular amino acid residue in the TCR-beta chain (PVR in the CDRs region of TCR-β) was conserved in different patients and also within the same patient before and after allo-SCT, and the PVR+ Tax-specific CTL clone selectively expanded in ATL long-term survivors as less-differentiated effector memory CTLs. Actually, the PVR+ CTL showed not only strong binding activity for the Tax-tetramer but also strong killing activity against patients’ HTLV-1-infected T-cells without any reaction against normal cells.

Aims: Currently, we are planning a redirected T-cell immunotherapy using the PVR+ TCR genes for ATL. Therefore, we prepared donor-derived PVR+ TCR-transduced T-cells and evaluated their cytotoxic efficiency against HTLV-1-infected T-cells and ATL-cells were both in vitro and in vivo mouse model.

Methods: HLA-A24-02 restricted and Tax301-309-specific TCR-α/β genes were cloned from an established PVR+ CTL clone and integrated into a retroviral siTCR 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-siCTLs). First, cytotoxicity and cytokine production capability of the Tax-siCTLs 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-siCTLs, the bioluminescence assay (in vivo imaging system) was performed. We generated a luciferase-gene transduced HLA-A24+HTLV-1 infected cell-line, MT-2 (Luc-MT-2), and injected 1×106 Luc-MT-2 cells into six-week-old NOD/Shi-scid, IL-2R-ko Gc (NSG) mice intraperitoneally. After the 3 weeks, 2×106 Tax-siCTLs were administered into the tail vein. In addition, western-blot analysis confirmed the stable expression of FANCA protein in the edited cells. Moreover reversion of the characteristic MNC hypersensitivity and restoration of the FANCD2 foci formation were observed in these cells. In addition, western-blot analysis confirmed the stable expression of FANCA protein.

Summary/Conclusions: Our results demonstrate for the first time that NHEJ gene correction is feasible in FA HSPCs. The high efficacy of the NHEJ repair approach clinically relevant for the future treatment of the hematopoietic defects in FA patients.

P624

NOVEL, ENHANCED AND DUAL TARGETING CAR INVARIANT NKT CELL-BASED IMMUNOTHERAPY FOR CD1D B CELL MALIGNANCIES
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Background: Anti-CD19 chimeric antigen receptor T cell (CART19) immunotherapy has shown promising clinical potential in relapsed/refractory mature B cell malignancies. However, only about half of patients benefit, highlighting the need for more effective CAR-based strategies. iNKT cells are rare regulatory cells that can activate innate and adaptive immune responses. They can efficiently induce a potent anti-tumor role. iNKT cells are restricted by CD1d, a non-polymorphic, phospho-glycolipid-presenting HLA I-like molecule. We previously showed that CD1d, as well as on normal B cells, is also expressed on malignant CD19+ B cells in mantle cell lymphoma (MCL), marginal zone lymphoma (MZL) and chronic lymphocytic leukemia (CLL).

Aims: We tested the hypothesis that bi-specific CAR/iNKT cells, targeting simultaneously CD19 and CD1D via the CD19-specific CAR and their natural invariant TCR respectively, might be more effective than CART19 cells against CD19+CD1D+ B cell malignancies.

Methods: We optimized a novel protocol for manufacturing 2nd (CAR2) and 3rd (CAR3) iNKT cells expressing CAR19. Their in vitro reactivity was assessed in cytotoxicity (flow cytometry-based) and cytokine and cytotoxic granule release assays (intracellular staining and Luminex technology). In vivo reactivity was assessed in NSG xenograft assays, with monitoring of 1T1C1D1 tumour cell growth and body weight.

Results: Our optimised protocol for selection, lentiviral transduction and clinical scale expansion of CARiNKT cells within 3 weeks is suitable for frozen and fresh lymphocytes, derived from either healthy donors or cancer, including lymphocytes from the bone marrow failure hematopoietic stem and progenitor cells (HSPCs) is the only current curative treatment for the bone marrow failure of patients with Fanconi Anemia (FA). However, the risks of GVHD and increased incidence of subsequent cancer, and the limited availability of matched donors hamper the adoption of this therapy in FA patients. For this reason correction of the FA-A patient-derived lym- phoblastic cell lines (CLLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELs generated in edited FA HSPCs in these cells. Methods: Two different FA mutations from FA-A patient-derived lymphoblastic cell lines (CLLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELs generated in edited FA HSPCs in these cells. Results: NHEJ-mediated gene editing approach can generate compensatory mutations that can restore the FA function in HSPCs from FA patients, mimicking reversion 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 HSPCs in these cells.

P623

NHEJ-BASED GENE EDITING: A NOVEL GENE THERAPY APPROACH IN FANCONI ANEMIA HEMATOPOIETIC STEM AND PROGENITOR CELLS
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Background: Allogeneic transplantation of hematopoietic stem and progenitor cells (HSPCs) is the only current curative treatment for the bone marrow failure of patients with Fanconi Anemia (FA). However, the risks of GVHD and increased incidence of subsequent cancer, and the limited availability of matched donors hamper the current application of this therapy in FA patients. For this reason correction of the FA-A patient-derived lym- phoblastic cell lines (CLLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELs generated in edited FA HSPCs in these cells. Methods: Two different FA mutations from FA-A patient-derived lym- phoblastic cell lines (CLLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELs generated in edited FA HSPCs in these cells. Results: NHEJ-mediated gene editing approach can generate compensatory mutations that can restore the FA function in HSPCs from FA patients, mimicking reversion 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 HSPCs in these cells.

Methods: Two different FA mutations from FA-A patient-derived lymphoblastic cell lines (CLLs) and primary HSPCs were targeted by the CRISPR/Cas9 system. INDELs generated in edited FA HSPCs in these cells. Results: NHEJ-mediated gene editing approach can generate compensatory mutations that can restore the FA function in HSPCs from FA patients, mimicking reversion 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 HSPCs in these cells.
phoma patients. The manufacturing process consistently allows high CAR transduction efficiency of CD4+ and CD8+ 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 CD1d and CD19 targets, demonstrated that CARiNKT19 cells are CD19-specific, retain their natural CD3ζ–CD28+ phenotype and exert additive dual-specific cytotoxicity against CD19+CD1d+ 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 CAR-T cells. Likewise, CAR2- and CAR3-iNKT cells are equally or more effective than their CAR-T counterparts in killing CD19+CD1d+ lymphoma and myeloma cell lines (B-lymphoblastoid 1CR1CD and lymphoma-derived Farage cells) and consistently more effective against primary MCL, MZL and CLL cells. Finally, in an in vivo NSG xenograft model of lymphoma, while survival of T- and NK cell-treated animals was the same as that of untreated animals (P=0.23), both CARiNKT19- and CAR-T cell-treated animals had significantly and comparably improved overall survival (P<0.001). However, compared to CAR-T, 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 CAR-T cells against CD19+CD1d+ 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 aGVHD 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 in target cells was confirmed by RNA-based RT PCR.

Results: Following transduction and puromycin selection ≥90% of CD3+ T cells expressed the Nkp44 CAR. In addition, most T cells displayed an effector-membrane phenotype. Upon co-culture with the B7H6 expressing targets such as K562 and HL-60 (myelogenous leukemia cell lines), NALM 16 (pre-B-ALL) and patient-derived AML samples (e.g. M2506 and M2987) Nkp30 redirected T cells elicited potent IFN-γ release and exhibited cytolytic activity to both leukemia lines and primary AML blasts in vitro. These responses were specific as e.g. no reactivity to the B7H6 negative myeloma line U266 was observed. We then evaluated the cytomorphologic response of Nkp30 redirected T cells in vivo. Upon adoptive transfer of Nkp30-CAR T cells into NSG mice engrafted with K562 significant reduction of tumor burden was observed. Moreover, injection of 1 - 5x10⁶ HLA-1III. Dept. of Medicine - Hematology, Internal Oncology & Pneumology, University Medical Center 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 T lymphocytes to combat CD19+ lymphomas have revealed compelling results. However, suitable antigens for an effective and specific CAR-mediated therapy to acute myeloid leukemia (AML) are still warranted as e.g. CD33 and CD123 CAR expressing T cells induce potent immune responses not only to AML blasts but also recognize normal hematopoietic stem cells (HSC). In contrast, B7H6, a member of the B7 family, is frequently expressed on various tumor cells including AML blasts while not detectable on normal tissues, and is recognized by the natural killer (NK) cell activating receptor NKp30. Moreover, NKp30 recognizes human leukocyte antigens (HLA)-B-associated transcript 3, a nuclear factor that is secreted and translocated to the cell surface in stressed and transformed cells.

Aims: In the current study, we thus explored the use of human T cells redirected to express a Nkp30-CAR for inducing effective antileukemic immunity in vitro and in vivo using a murine model of lymphoma. We also performed functional dissection showed that activated CART19 cells 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 TRANSFER IN COMBINATION WITH COMPLEMENTARY INHIBITORS 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 underscores the need for an improved understanding of underlying factors limiting the efficacy of T-cell-based immunotherapy and its wide application.

Aims: We explored an immunotherapeutic combination strategy to unleash the full power of adoptively transferred antigen-specific T cells. We also tested the hypothesis that targeting the BAFF-R molecule with the immune-stimulatory cytokine IL-2 would result in a more sustained antitumor effect.

Methods: Human T cells from healthy donors were retrovirally transduced with MDM2- and p53-specific TCRs and expression levels were analyzed by flow cytometry. MDM2 and p53 protein expression in MM cell lines was determined by Western blot. The therapeutic efficacy of adoptive TCR transfer was evaluated in NOD-SCID IL2R-gamma-chain(null) (NSG) mice engrafted with human melanoma cells expressing either MDM2 or p53. Tumor growth and intratumoral alterations were monitored and intratumoral effector function was determined by IFN-gamma ELISPOT assays.

Results: T cells expressing the MDM2-specific TCR showed a superior anti-tumor response in vivo compared to single TCR treatment, demonstrating the need to target multiple MM antigens to circumvent tumor escape mechanisms associated with down-regulation of antigen. Yet, we observed a strong up-regulation of PD-L1 expression in tumor cells in vivo and in vitro in T cells expressing MDM2-specific TCRs. To overcome this PD-L1-mediated resistance, we performed combination experiments with anti-PD-1-neutralizing antibodies. In vivo ACT experiments combined with anti-PD-1 inhibitor demonstrated the synergistic therapeutic potential of this approach compared to single agent. Yet, it does not result in complete tumor eradication suggesting that targeting one single immune checkpoint receptor is not sufficient to drive a full anti-tumor response.

Summary/Conclusions: Combination inhibitor checkpoint approach has demonstrated potential in our ACT experimental MM model and forms the basis for a novel multi-modal immunotherapeutic combination treatment for multiple myeloma.

P628 ENGINEERED T CELLS TOWARDS BAFF RECEPTOR: A NOVEL STRATEGY TO EFFICIENTLY TARGET B-CELL ACUTE LYMPHOCYTIC LEUKEMIA N. Turazzi1,2, G. Fazio1, V. Rossi1, A. Rolink1, G. Cazzaniga1, A. Biondi1, C. F. Magnani2, E. Biagi1

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Background: B-cell Acute Lymphoblastic Leukemia (B-ALL) is most common in children (80%), but it has also a peak of incidence in adult age. Immunotherapeutic approaches targeting the CD19 molecule paved the way for the treatment of relapsed and refractory lymphoblastic leukemia, which remains a major therapeutic challenge. Recently, the emergence of relapses with CD19-epitope loss in 10-30% of treated patients has been reported. This newly identified escape mechanism has been recently shown to be related to the combination of deleterious mutations and emergence of alternatively spliced RNA isoforms, as effect of selective pressure.

B-cell Activating Factor (BAFF) Receptor is a transmembrane protein which is fundamental for B-cell maturation and survival. Moreover, the expression of this receptor is restricted to mature B cells and, interestingly, is not present on bone marrow B-cell precursors. Recent studies reported the over-expression of BAFF Receptor (BAFF-R) in various B-cell malignancies such as B-ALL, B lymphoma, chronic lymphocytic leukemia and myeloma. In the context of B-ALL-leukemic cells express both BAFF-R and B-cell receptor suggesting the presence of an autocrine signalling loop. BAFF is also expressed in bone marrow microenvironment by endothelial cells which support the proliferation and the survival of primary B-ALL blasts.

Aims: In the current study, we aimed to develop a chimeric antigen receptor (CAR) approach targeting the BAFF-R molecule.

Methods: We characterized the expression of BAFF-R in B-ALL primary samples. As immunotherapeutic approach to target BAFF-R molecule, we developed six anti-BAFF-R.CARs that differ for the inversion of the VH and VL and the length of the spacer domain.

Results: Six anti-BAFF-R.CARs expressed in B-ALL primary samples at the onset and relapse. In order to develop a chimeric antigen receptor (CAR) approach targeting BAFF-R molecule, six anti-BAFF-R CAR genes that differ for the inversion of the VH and VL and the length of the spacer domain have been generated. Cytokine-induced Killer (CIK) cells, engineered using an improved Sleeping Beauty (SB) transposition system, stably expressed anti-BAFF-FR.CARs, and maintained their characteristic phenotype. Among the newly constructed CARs, the shortest VH VL anti-BAFFR CAR exerted the highest anti-leukemic activity towards target cells, such as NALM-6, with an in vitro killing activity >80% of the effector to target ratio (E:T). Noteworthily, CAR mediated cytotoxicity if measured by detection of cytotoxic release by intracellular staining (8.9±2.6% of IFN-g and 16.4±5.5% of IL-2 producing cells). Importantly, we also detected a specific cytokytic activity towards primary B-ALL blasts (average 65.6±5.4%, n=9). Combining the iNKT sh.CAR with CD19.CAR we detected a superior antitumor activity towards ALL targets (at least 3 E:T ratios resulted in >72% cell lysis) and 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 iNKT sh.CAR to lyse CD19-negative blasts.

Summary/Conclusions: Taken together, these findings make this receptor a suitable target for a second-line B-ALL immunotherapy in case of relapse after CD19-targeting therapies or for a double targeted approach. Being restricted to mature B cells, but absent on precursors and plasmablasts, our strategy could have an inferior toxicity concerning the emergence of B-cell aplasia observed in patients treated with anti-CD19 CAR-modified T cells.

P629 EXPLORING HUMAN TCR- AND CAR-REDIRECTED INKT CELLS FOR ADOPTIVE CELLULAR THERAPY B. Mir1, S. Khan1, M. Theobald1, U. Hartwig1,2

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Background: T-cell receptor (TCR) - or chimeric antigen receptor (CAR) re-directed T cells have substantially improved adoptive cellular therapy (ACT) for solid or hematological malignancies. T cells equipped with effectors arms are able to target tumors and have inherent antitumor 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 ACT.

Aims: In the current proof 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 healthy donors using Vβ11- (CD81-) conjugated, anti-INKT microbeads (Miltenyi Biotec) and expanded in vitro upon coculture with autologous DCs loaded with α-galactosyceramide (α-GalCer) loaded DCs. β2m and 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 the percentage of redirected cells were performed by flow cytometry functional activity of human INKT cells was assessed by killing of CD19+ NALM-16 (pre B-ALL) and primary AML (M2653) cells as targets.

Results: Following isolation of 0.7 – 0.8 x 10^6 Vβ24,Jα18/Vβ11+ INKT cells from PBMC we achieved on average a 120-fold expansion 21-28 days after stimulation with α-GalCer loaded, irradiated autologous DC and 25 U 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 such as CD56 and CD16. Following retroviral transduction and selection for 6 days >80% of TCR (5B2)- and CD19 CAR-redirected INKT cells were obtained. 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-g and elicited potent antileukemic responses to AML M2653 and NALM-16 in vitro. Studies to examine their cytokitic potential within in vivo using NSG xenograft models are currently in progress.

Summary/Conclusions: These studies demonstrate that purified human Vα24/Jβ11+ INKT cells expanded from PBMC can be successfully redirected against leukemia both in vitro and in vivo 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.
Background: Acute Myeloid Leukemia (AML) is an aggressive malignancy still associated with high relapse rates when treated with conventional chemotherapeutic and hematopoietic transplantation regimens. In search for alternative strategies, interest has focused on antigen-specific immunotherapies and in particular on T cells redirected with Chimeric Antigen Receptors (CARs) that have shown exciting results in cancer therapy, especially in the context of B-cell malignancies. CD33 is the only validated target in AML so far and represents a suitable antigen to be targeted with CAR-T cells, being broadly expressed on AML blasts.

Aims: The aim of the present study is to preclinically evaluate the efficacy and safety profiles of CD33 CAR redirected Cytokine Induced Killer (CIK) cells alone and in combination with standard chemotherapeutic agents.

Methods: Here we proved the feasibility of harnessing Cytokine Induced Killer (CIK) cells 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-CK 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 CICK cells is evaluated in NSG mice transplanted with AML cell lines (M4A-NRas cells) and primary samples.

Results: CD33.CAR-CK 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 M4A-NRas cells grafted mice with the already established 5+3 induction chemotherapy protocol, we confirmed that chemotherapy is able to significantly reduce the leukemic burden from around 20% to 0.1% in the bone marrow. Since the AML disease is not totally eradicated, this model will be therefore suitable for further investigation of 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-CK 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 myelootoxicity due to the long-term off-target effect on normal hematopoietic stem/multioid progenitor cells. Finally, if successful, our results will provide the preclinical validation of CD33.CAR-CIK cell immunotherapy, supporting it development to the clinic.

P631

UPDATE ON THE FIRST PATIENTS WITH SEVERE HEMOGLOBINOPATHIES TREATED WITH LENTIGLOBIN GENE THERAPY

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Background: The lentiviral vector LentiGlobin (DP) uses an autologous globin gene (HbAT87Q) to overcome genetic mutations affecting HbA and HbS. The technology is designed to induce fetal-type hemoglobin expression in primary hematopoietic stem cells (HSCs) that could reduce or eliminate symptoms of severe sickle cell disease (SCD) and transfusion requirements in transfusion-dependent β-thalassemia (TDT).

Methods: Aims: The first patients treated with LentiGlobin received 200-250 mg/m² intravenous 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: A single patient with severe SCD (male; 13 years old) and 4 patients with TDT (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) x10^9 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 patients 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 myeloablative 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 HbA1c (48%) and 6.5 g/dL sickle Hb (HbS; 50%); in addition, their unconjugated bilirubin, lactate dehydrogenase and reticulocyte count had dropped by 50%, 55%, 26%, respectively, compared to screening. Of the 4 patients with TDT, 3 have β0/βE genotypes and 1 is homozygous for a severe β0 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 HbA1c 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 HbA1c 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 HbA1c 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 myeloablative conditioning. Follow-up data on the 5 previously reported patients and early results from the 2 recently treated patients will be presented.
Indolent Non-Hodgkin lymphoma - Clinical

P632
A SINGLE INSTITUTIONAL EXPERIENCE OF 261 PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

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Background: Large granular lymphocytic leukemia (LGLL) is a rare clonal lymphoproliferative disorder of post-thymic T-cell or natural killer (NK)-cell lineage associated with 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 27.2% 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 a 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 was observed (p=0.079) to any immunosuppressive therapy was noted. There was a 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/μL (p=0.0078), bone marrow involvement (p<0.0001), and splenomegaly (p<0.0001).

Summary/Conclusions: In this large retrospective study, we described the frequency of LGLL-associated manifestations and their impact on the course of LGLL. Severe anemia/transfusion dependence, lower LGL counts, bone marrow involvement, and splenomegaly were suggestive of more aggressive disease. We confirmed that there is no difference in overall survival among first line immunosuppressive therapies.

P634
ONGOING PHASE 1/2 STUDY OF INCB050465, A SELECTIVE PI3K-Delta INHIBITOR, FOR THE TREATMENT OF PATIENTS WITH RELAPSED/RECURRENT B-CELL MALIGNANCIES (CITADEL-101)

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Background: Signaling networks mediated by PI3Kδ have been implicated in proliferation, migration, and functioning of B-cells. INCB050465 is a novel, potent, and selective inhibitor of PI3Kδ (IC50 >250 nM for more selective for PI3Kδ vs other isoforms). INCB050465 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 INCB050465 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), nor- mal 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 INCB050465 5mg QD. Subsequent cohorts used a 3+3 design and evaluated doses of 10–45mg QD. Based on PK/pharmacodynamics, the 20 and 30mg QD cohorts were expanded. Responses were assessed every 8 weeks using the Lugano Classification or International Working Group on Chronic Lymphocytic Lymphoma (CLL) criteria.

Results: As of the data cutoff (Nov 1, 2016), 52 patients were treated (median age 65 years, range 18-88). 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 months (range, 0.6-13.4); no DLTs were identified. Sixty-seven percent of patients discontinued therapy, most commonly due to disease progression (31%) and AEs (25%). Thirty-three percent of patients had dose interruption and 4% had dose reduction. Most common nonhematologic AEs (all 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, INCB050465 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. Differen doses regimens/schedules, long-term safety, and disease-specific cohorts are being evaluated.
lymphoma (IFL). Upon informed consent, patients receive 12 cycles of R2 induction chemotherapy followed by 21 of 28 (1 cycle every 3 weeks) and prednisone (75 mg/m²) every 3 weeks, 191.92% and 191.92% of patients achieved a 21% response rate (95% CI) to CT-P10 compared to R10 in COOP (P < .001). Results: At the 191.92% and 191.92% of patients achieved a 21% response rate (95% CI) to CT-P10 compared to R10 in COOP (P < .001). Results: At the
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 achieving high rates of complete clearing and durable responses (median TTNT not reached) with only 38% of patients requiring 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 provides a longer TTNT than PUVA or IFN-α monotherapy (36.3 months and 14.0 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 OF BRENTUXIMAB VEDOTIN (BV) OR PHYSICIAN’S CHOICE (PC) OF METHOTREXATE (MTX) OR BEXAROTENE (BE) IN CD30-POSITIVE CUTANEOUS T-CELL LYMPHOMA (CTCL): NUMBER NEEDED TO TREAT ANALYSIS

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2Department of Medicine, Mayo Clinic, Rochester, MN, United States, 3University Hospital of Basel, Basel, Switzerland, 4Memorial Sloan Kettering Cancer Center, New York, United States, 5University of Texas MD Anderson Cancer Center, Houston, TX, United States, 6Stanford University of Medicine and Stanford Cancer Institute, California, United States, 7University Hospital Zürich, Zürich, Switzerland, 8University Hospital Birmingham, Birmingham, United Kingdom, 9University of Turin, Turin, Italy, 10University of Bologna, Bologna, Italy, 11University Hospital Leuven, Leuven, Belgium, 12Columbia University, New York, United States, 13Seattle Genetics, Inc., Bothell, United States

Background: CTCL is a generally incurable, relapsing disease associated with a significant symptom burden, including disfiguring lesions, debilitating pruritus and frequent skin infections. ALCANZA is a Phase 3 study of BV vs PC (MTX or Bex) for the treatment of CD30-positive (CD30+) CTCL (NCT01578499). BV was associated with significantly improved rate of objective response lasting ≥4 months (ORR4; 56% vs 13%; p<0.0001), longer median progression-free survival (PFS; 16.7 vs 3.5 months; p<0.0001), and decreased symptom burden measured by Skindex-29 (27.96 vs –8.62; p<0.0001), compared with PC. BV’s safety profile was consistent with previous reports, with all-grade and grade 3 peripheral neuropathy of 67% and 9%, respectively. Number needed to treat (NNT), defined as the number of patients (pts) that need to be treated to prevent one disease progression event or death compared with placebo.

Methods: The NNT with BV was calculated as the inverse of the absolute risk reduction (ARR); ARR was the PFS event rate per independent review facility (IRF) assessment in the PC arm minus the event rate in the BV arm. PFS was defined as the time from randomization until progressive disease/death due to CTCL (International Working Group [IWG] criteria). The NNT event was death. The NNT event was death. PFS was defined as the number of patients (pts) that need to be treated to prevent one outcome event relative to the comparator therapy, is an effective method for comparing the efficacies of interventions. A number needed to treat (NNT) of 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.

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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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1Department of Hematology, St. Mary Hospital, Seoul, Catholic Medical Center, Seoul, Korea, Republic Of
2Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea
3Department of Ophthalmology, Hallym University College of Medicine, Chuncheon, Korea

Background: Although the recent reports show that interest in ocular adnexal lymphomas (OAL) and their biological 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 little comparative study of other 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 of college (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, status of BM involvement, primary therapeutic modalities, response to initial therapy, and treatment-related complications with survival outcomes.
P639
CLONAL B-CELL LYMPHOCYTOSIS OF MARGINAL ZONE ORIGIN (CBL-MZ): A PROSPECTIVE REGISTRATIONAL STUDY ON 96 CASES

C. Kalpadakis1,2, G. Pangalis2, T. Vassiliakopoulos3, S. Sachanas2, M. Moschogianis1, X. Yiallourou1, M-C. Kyrtoris2, P. Tsaknidis2, A. Dimitrakopoulou1, P. Korkolopoulou1, F. Kontopoulou2, E. Koulieris2, C. Pontikoglou1, M. Ximenis1, M. Roumelioti4, D. Rontogianni5, H. Papadaki1, P. Panagiotidis4, M. Angelopoulou3
1Department of Haematology, University Hospital, University of Crete, Heraklion, 2Department of Haematology, Athens Medical Center-Psychikon Branch, 3Department of Haematology, 41st Department of Propedeutics, University of Athens, Athens, Greece

Background: Clonal B-cell lymphocytosis of marginal zone origin (CBL-MZ) has been recognized as a provisional entity in the WHO classification. Despite diagnostic similarities with SMZL, the exact relation between them has not been established yet. AIM: To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.

Aims: To describe the main features of CBL-MZ and to further elucidate its relation to SMZL.

Methods: 96 CBL-MZ were analyzed. Staging at diagnosis included CBCs, blood morphology and immunophenotype, biochemistry, viral test for hepatitis C and B, serum immunoglobulin levels and immunofixation as well as whole blood morphology and immunophenotype, biochemistry, viral test for hepatitis C and B, serum immunoglobulin levels and immunofixation, as well as whole body CT scan. BM biopsies were available in 78 cases which were studied with the following panel of moAbs: CD20, DBA44, CD23, CD5, CD25, CD38, CD27, s/cIgM/D, TCL-1, MNSA, T-bet and IRA1. Gastroscopy with multiple biopsies was performed in 58 cases. FISH analysis for del1q was done in 13 pts, and detection for MYD88 mutation in 60.

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<td>De/iQ of progression</td>
<td>76 (80%)</td>
</tr>
<tr>
<td>De/iQ of treatment</td>
<td>76 (80%)</td>
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<td>De/iQ of outcome</td>
<td>76 (80%)</td>
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<td>De/iQ of 5-year</td>
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<tr>
<td>De/iQ of 10-year</td>
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REAL-WORLD EXPERIENCE WITH RITUXIMAB-FLUDARABINE (RF) AND DEXAMETHASONE, RITUXIMAB, CYCLOPHOSPHAMIDE (DRC) IN WALDENSTROM MACROGLOBULINEMIA: A RETROSPECTIVE STUDY FROM 163 PATIENTS
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Background: symptomatic Waldenstrom macroglobulinemia (WM) may be managed with various regimens. After the 8th IWWM congress, experts recommended the use of rituximab-based regimens. Two phase II trials treated DRC (n=72, 1L) and RF (n=43, one third having received chlorambucil alone, +/- steroids/rituximab before), with published long-term follow up of 95 and 46 months, respectively. Mature data from larger cohorts confirming trials’ results in real-life practice are lacking.

Aims: to evaluate RF or DRC in first-line WM treatment. We present here our real-world experience of 163 WM patients treated with DRC or RF from January 2005 to December 2015.

Results: median age of patients at diagnosis was 68.6y and at therapy 71.2y. Our cohort included 163 patients, 43 RF and 120 DRC, median age at diagnosis 68.6y and at therapy 71.2y. 75% were above 65y at diagnosis, 68.6% and at therapy 71.2y, respectively. Previous CLB had no impact on outcomes, but dose reductions >20% adversely impacted TTNT. IPSS scoring system did not improve prognosis. Long-term follow-up (22% of patients) showed that 70% of patients were still alive, 48% had no disease progression (median follow-up 6 years). Significant differences between DRC/RF cohorts were: median age 74/64y, high IPSS score 63%/28%, 2B2M/3mg/l 74%/56%, DRC cohort: median PFS/Time To Next Therapy and Overall Survival were 33m, 45.8m and 78 at 5 years, respectively. Previous CLB had no impact on outcomes, but dose reductions >20% adversely impacted TTNT. IPSS scoring system did not improve prognosis. Long-term follow-up (22% of patients) showed that 70% of patients were still alive, 48% had no disease progression (median follow-up 6 years).

Conclusions: Our real-world experience of 163 WM patients treated with DRC or RF showed that 70% of patients were still alive after 6 years and that dose reductions >20% adversely impacted TTNT. IPSS scoring system did not improve prognosis. Long-term follow-up (22% of patients) showed that 70% of patients were still alive, 48% had no disease progression (median follow-up 6 years).

P642

MICAFUNGIN VERSUS LIPOSOMAL AMPHOTERICIN B FOR EMPIRICAL ANTIFUNGAL THERAPY IN FEBRILE NEUTROGENIC 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, who remain febrile despite 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 (89.4%), p=0.855*), (4) absence of premature study drug discontinuation due to poor efficacy or drug-related adverse events (54/72 cases (75.0%) vs 47/66 cases (71.2%), p=0.615*), and (5) resolution of fever during neutropenia (45/72 cases (62.5%) vs 33/66 cases (50.0%), p=0.258*). However, discontinuation due to drug-related adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) vs 9/66 cases (13.6%), p=0.006*). In safety evaluation, adverse events of creatinine increase and hypokalemia were less often in the MCFG group (1.4% vs 9.0%), and ed adverse events occurred less frequently in the MCFG group (1/72 cases (1.4%) vs 5/66 cases (7.7%), p=0.115*).

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), 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 dichrotometer 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 led to enhanced activation (CD62L: 44% +/- 8 vs 13 +/- 2, *), mean +/- SEM, p value 0.05 considered to be significant. In vivo, Pretreatment with POS led to increased ROS (2868 +/- 4432 vs 8528 +/- 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 (6890 +/- 1338 vs 28730 +/- 6893, *). 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 +/- 3286 vs 41180 +/- 2584, zymosan, *), and IL-8 synthesis were substantially impaired. CAS showed an increased phagocytosis (50% vs 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. 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: Different antifungal drugs affect clinical course IPA in vivo. CAS shows variable modification with respect to 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 LEUKAEMIA CLASSIFIED ACCORDING TO EORTC/MSG CRITERIA OF INVASIVE FUNGAL INFECTION: A PROSPECTIVE STUDY BY THE RLTG OF THE RESEARCH COMMITTEE, MILAN

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1Hematology, Spedali Civili, Brescia, 2Hematology, Ospedale Niguarda, Milan, 3Fondazione IRCCS Policlinico S. Matteo, Pavia, 4Hematology, Ospedale di Circolo e Fondazione Macchi, Varese, 5San Raffaele Scientific Institute of Milan, Milan, 6Humanitas Cancer Center, Rozzano-Milano, 7Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, 8Hematology, Ospedale Valduce, Como, 9Hematology, San Gerardo Hospital, Monza, Italy

Background: In acute leukemia (AL) patients (pts) pulmonary infections may be severe and worsen the final outcome of AL. They have been recently shown to adversely affect the outcome of bloodstream infections (BSI) in AL pts (Cat- be severe and worsen the final outcome of AL. They have been recently shown to adversely affect the outcome of bloodstream infections (BSI) in AL pts (Cat-

Aims: In order to better define the clinical and prognostic significance of PI in AL pts in a real-life setting, we have analyzed all PI diagnosed during consecutively conducted 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 by cyclophosphamide (cpm) 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 PI (15.9% and 10%), but significantly higher in prob/prov PI (31.3%, p<0.0192). Multivariate analysis confirmed a prob/prov PI (3.277, 1.243-8.644) predictive for death, as well as relapsed/refractory AL (2.45, 1.092-5.498) and BSI (2.833, 1.056-5.377).

Table 1.
**P645**

**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 centers.

**Aims:** The purpose of this study is to report our single center experience of surgical interventions for IFI in acute leukaemia patients.

**Table 1.**

<table>
<thead>
<tr>
<th>Number of surgical procedures</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Organ involved</td>
<td>1 ilio-pelvic lymph node, 1 diaphragm, 2 iliac, 1 oesophagus, 1 diaphragm, and 1 lung (organ)</td>
</tr>
<tr>
<td>Involvement</td>
<td></td>
</tr>
<tr>
<td><strong>Types of Surgery</strong></td>
<td>11</td>
</tr>
<tr>
<td>2. Thoracotomy with decortication</td>
<td>1.5, 2.5, 2.5, 2.5</td>
</tr>
<tr>
<td>3. VATS with decortication</td>
<td>2.5, 2, 2</td>
</tr>
<tr>
<td>4. VATS with decortication and upper lobe resection</td>
<td>2, 2, 2</td>
</tr>
<tr>
<td>5. VATS with decortication and lower lobe resection</td>
<td>2, 2, 2</td>
</tr>
<tr>
<td>6. Open thoracotomy</td>
<td>1, 2, 2, 2</td>
</tr>
<tr>
<td>7. Abdominal dissection</td>
<td>1, 1, 1, 1</td>
</tr>
<tr>
<td>8. Multiple procedures (for drainage of multiple abscesses)</td>
<td>1, 1, 1, 1</td>
</tr>
<tr>
<td>9. Thoracotomy for empyema and abscess</td>
<td>1, 1, 1, 1</td>
</tr>
</tbody>
</table>

**Complications:**

- Aspergillus pneumonia
- Uncontrolled bleeding
- Aspergillus tracheal involvement
- Lungs
- Aspergillus paracancer

**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%). An and an IgG-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 222 chest images did not have 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). High-dose chemotherapy was noted in 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 experienced 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-
Background: Neutrophenic 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 one of the lectin pathway components of the lectin pathway, 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, rs1522306519, rs1840880) 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-ficolin SNPs were identified as risk factors for developing sepsis and/or pneumonia. Patients harboring rs17514136GG (OR = 2.39; 95% CI: 1.0–5.64) or rs1522306519CC (OR = 2.35; 95% CI: 1.05–5.26) were at higher risk of developing sepsis and/or pneumonia compared to patients harboring the other genotype combinations. In the PTX3 gene, the A17263G (rs17549193) was found to be associated with an increased risk of developing sepsis and/or pneumonia. The risk of developing sepsis and/or pneumonia was higher in patients with the A17263G (G/G) genotype compared to the A17263G (A/G) and A17263G (A/A) genotypes (OR: 2.52; 95% CI: 1.19–5.33, p = 0.015).

Summary/Conclusions: This study is the first to investigate the impact of genotypes of L-ficolin and PTX3 on the occurrence of infectious events in adult patients with newly diagnosed AML following anthracycline-based induction chemotherapy. Further studies are needed to confirm these findings and to investigate the potential mechanisms underlying the observed associations.

P650
TIMING OF DEFIBROTIDE INITIATION POST-DIAGNOSIS OF HEPATIC VENO-OCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME AFTER PRIMARY CHEMOTHERAPY: EXPLORATORY ANALYSIS OF AN EXPANDED-ACCESS PROTOCOL

Background: Hepatic VOD/SOS, which may be unpredictable and potentially life-threatening, is typically considered a complication of hematopoietic stem cell transplantation (HSCT); however, VOD/SOS can occur after chemotherapy without HSCT. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Defibrotide is approved to treat severe hepatic VOD/SOS post-HSCT in the European Union and to treat hepatic VOD/SOS with renal or pulmonary dysfunction post-HSCT in the United States.

Aims: To perform an exploratory post hoc analysis of the impact of timing of initiation of defibrotide after VOD/SOS diagnosis in patients developing VOD/SOS after primary chemotherapy without HSCT (off label).

Methods: In an expanded-access protocol for patients with VOD/SOS post-HSCT or chemotherapy, with or without MOD (renal and/or pulmonary dysfunction), defibrotide 25mg/kg/d (4 divided doses of 6.25mg/kg) was given a median of 1 day (range: 0–17 days) from VOD/SOS diagnosis to start of defibrotide. In the post-chemotherapy subgroup, survival was analyzed post hoc from the day VOD/SOS was diagnosed (days 0–30 after start of chemotherapy) through follow-up, which was collected for 100 days post-chemotherapy. For these exploratory analyses, survival rates in the post-chemotherapy subgroup were estimated. Time from VOD/SOS diagnosis to start of defibrotide for (1) all patients before/after days 1, 2, and 4, 7, 14, and using Fisher’s exact test and (2) patients starting defibrotide on a particular day: 0, 1, 2, 3, 4, 5, 6, 7, 8–14, and ≥15, by Cochran-Armitage test for trend across days. Causes of treatment delay were not assessed.

Results: In the final dataset, 137 patients developed VOD/SOS after primary chemotherapy. Of these, 87 patients (41 with MOD) developed VOD/SOS by day 30 after the start of chemotherapy. In the latter group, 79.3% (69/87) were aged ≤16 years. In 26.4% (2387) of post-chemotherapy patients, defibrotide was started the day of diagnosis; in 89.7% (78/87), by Day 7. In the population with defibrotide initiation post VOD/SOS, mean number of days from diagnosis to the initiation of defibrotide in both the overall group and MOD subgroup (Figure), earlier initiation was associated with higher Day +100 survival rates for all days, which was significant at a number of timepoints. The trend test for particular initiation days was statistically significant for each post hoc analysis.
also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall group and MOD subgroup (P<.05). In the overall post-chemotherapy population, adverse events (AEs) and serious AEs occurred in 66% and 40% of patients, respectively. Aside from multi-organ failure, the most common AE of any severity was hypotension (9.5%). Possibly related AEs lead to discontinuation in 7.3%, most common was gastric hemorrhage (3.7%).

**Summary/Conclusions:** In this exploratory analysis of final study data in the subgroup of patients developing VOD/SOS after chemotherapy, earlier defibrotide initiation post-VOD/SOS diagnosis was associated with improved Day +100 survival, confirmed by the Cochran-Armitage test (P<.05), even in the small MOD subgroup. This time-dependent relationship was consistent with that found in the HSCT subgroup from this study. No specific day appears to provide a clinically meaningful cutoff for better Day +100 survival, suggesting that later intervention retains value if treatment must be delayed.

**Support:** Jazz Pharmaceuticals

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**ADAMTS-13 REGULATES NEUTROPHIL RECRUITMENT IN A MOUSE MODEL OF INVASIVE PULMONARY ASPERGILLOSIS**

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**Background:** Von Willebrand factor (VWF) is produced as multimers of various sizes and is secreted as an acute phase protein during inflammation. The main mechanism regulating the size and prothrombotic activity of VWF is the specific proteolytic activity of ADAMTS-13 (a disintegrin and metalloprotease with ThromboSpondin type 1 repeats-13) which is diminished under several pathological conditions.

**Aims:** To determine the relevance of this regulatory pathway for the innate inflammatory response by polymorphonuclear neutrophils (PMN), we employed a mouse model of invasive pulmonary aspergillosis (IPA) where PMN functionality is crucial for fungal clearance and survival.

**Methods:** IPA was induced by intratracheal application of *Aspergillus fumigatus* (*A. f.*) conidia in wildtype (129/Sv/Pas) or ADAMTS-13 deficient (*Adamts13*−/−) mice, and VWF deficient (*Vwf*−/−) mice or respective controls (B6). Some mice were sacrificed 24 h after infection. Fungal load was assessed as colony forming units (CFU) after plating and culturing lung homogenates on Sabouraud agar plates. For histological analysis paraffin sections of the lungs were stained with H&E, mouse complement component C3d and VWF antibody. Broncho alveolar lavage fluid (BALF) was analyzed for cell count (bead-based by flow cytometry or by an animal blood counter), ELISA was performed for albumin amount and cytokines were analyzed by a multiplex assay. Bone marrow-derived PMN were isolated by magnetic cell sorting using biotin labeled Ly6G/C specific antibody. PMN functions were analyzed for degranulation, oxidative burst activity and CD62L shedding by flow cytometry. Fungal killing of PMN in *vitro* was assessed by a XTT assay. Chemotactic properties of *A. f.*-activated and control serum from wildtype and knock-out mice was evaluated by migration of purified human PMN, isolated by dextran sedimentation and Histopaque® centrifugation, in a transwell assay.

**Results:** While infected neutropenic mice developed lethal IPA, all wildtype mice survived the infection. Interestingly, *Adamsats13*−/− mice displayed more severe signs of disease with a lethal course in about 24% of the animals. Examination of the lungs revealed a higher fungal burden along with increased signs of acute lung injury and levels of pro-inflammatory cytokines in ADAMTS-13 deficient mice. Histology sections demonstrated a more pronounced perivascular leukocyte infiltration in support of a dysregulated inflammatory response in *Adamsats13*−/− mice. Importantly, we observed no general defect in the activation of neutrophil effector functions in response to conidia or hyphae *in vitro*. Furt-
Myelodysplastic syndromes - Biology

P652
IDENTIFICATION OF THE SPECIFIC HEMATOPOIETIC STEM CELL POPULATIONS RESPONSIBLE FOR FAILURE TO HYPMETHYLATING AGENTS IN MYELODYSPLASTIC SYNDROMES
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Background: Myelodysplastic syndromes (MDS) are hematopoietic disorders characterized by the ineffective production of mature blood cells of one or more lineages and by the risk of evolution to acute myeloid leukemia. The current standard of care for MDS patients is the treatment with hypomethylating agents (HMA); however, response to drugs from this family occurs in just about half of the patients and is accompanied by high rates of therapy failure. Failure to HMA in MDS is a poorly understood process associated to increased risk of disease progression and to a dismal prognosis and cannot be, thus far, predicted or prevented.

Aims: Given that MDS are stem cell disorders, our aim was the identification and molecular characterization of the specific hematopoietic stem/progenitor cell (HSPC) population in which the relapse-driver clones arise. This is an essential step for the development of effective monitoring and early intervention protocols for HMA failure.

Methods: Using flow cytometry immunophenotyping, we quantitatively analyzed the different cell subpopulations within the CD34+CD38− and CD34+CD38+ HSPC compartments in 122 sequential MDS bone marrow samples obtained from 93 patients at different stages of HMA treatment.

Results: In line with earlier reports suggesting the presence of alterations in myeloid progenitor frequencies in MDS, our flow cytometry data stratified untreated patient samples in two groups representative of two abnormal differentiation patterns, which were independent of the IPSS risk classification. The “CMP pattern” group (12 samples, 34%) was characterized by an increased frequency of the common myeloid progenitors (CMP) (2.6-fold; p<0.04) whereas the “GMP pattern” group (23 samples, 66%) was characterized by the increased frequency of the granulomonocytic progenitors (GMP) (1.7-fold; p<0.04) within the HPC compartment, when compared to those from healthy individuals. Importantly, these two patterns were not caused by the expansion of the aforementioned populations, but by the depletion of other progenitors within the same compartment, which points to the existence of selective differentiation defects in upstream HSC or multipotent progenitors (MPP). For instance, decreased numbers of long-term (LT)-HSC (-5.1-fold, p=0.001) and increased counts of the GMP precursors, lymphoid-primed multipotent progenitors (LMPP) (4.7-fold, p=0.001), were observed in CMP pattern patients but not in CMP pattern patients. These data suggest that each abnormal differentiation pattern arises from defects in different HSC populations and has a differential impact in the number and functionality of downstream progenitor cells (R. Beran1, D.-C. Liang2, M.-C. Chiu1, Y.-J. Huang1, L.-Y. Shih3
1Chang Gung Memorial Hospital, Taoyuan, 2Mackay Memorial Hospital, Taipei, 3Chang Gung Memorial Hospital and Chang Gung University, Taoyuan, Taiwan, Republic of China

Background: Our previous studies showed that RUNX1 and ASXL1 mutations were frequently co-existed in chronic myelomonocytic leukemia (CMMML) (EHA 2015) and clonal evolution of RUNX1 and/or ASXL1 occurred most frequently in chronic myeloid leukemia (CML) with myeloid blast crisis (EHA 2016). The molecular pathogenesis of cooperation of RUNX1 and ASXL1 mutations has not been reported yet.

Aims: We aimed to determine the functional role of collaborative association of RUNX1 and ASXL1 mutations for secondary acute myeloid leukemia (sAML) transformation.

Methods: For in vitro study, we overexpressed RUNX1-WT/MT (R135T) in K562 cells which harboring ASXL1-MT (Y891X) and co-expressed with ASXL1-WT/MT (R693X) in murine 32D cells. After stable expression, functional properties were examined by using immunoblot, co-immunoprecipitation, quantitative RT-PCR, flow cytometry, cell proliferation, colony formation and gene expression microarray analyses. C57BL/6 mice were used for bone marrow transplantation (BMT) experiments for in vivo study.

Results: We found that RUNX1-MT augmented cell proliferation, colony formation, HOXA gene expression and inhibited megakaryocytic differentiation in ASXL1-MT K562 cells compared to RUNX1-WT or empty vector control. The cooperation of RUNX1 and ASXL1 mutations or the knocked down of ASXL1 cooperated with RUNX1-MT inhibited apoptosis and impaired differentiation in 32D cells. Nine months post BMT mice with the combined RUNX1 and ASXL1 mutations, but not RUNX1-MT or ASXL1-MT alone, developed disease characterized by marked splenomegaly, hepatomegaly, and leukocytosis with a shorter latency. We found that RUNX1-MT stabilized hypoxia-inducible factor 1α (HIF1-α) 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 controlled to sample patients. We also examined the impact of RUNX1 and ASXL1 mutations on sAML-free survival of 104 Patients with CMMML in whom 11 had co-occurrence of RUNX1 and ASXL1, 39 had either mutated ASXL1 or RUNX1 and 54 patients were negative for both mutations. We found that patients carrying co-existed mutations had a shorter sAML-free-survival (median 16.1 months, 95% CI 0.0-60.1 months) than those carrying either mutated gene alone (median 23.0 months, 95% CI 17.8-28.2 months) or negative for both mutated genes (median not reached, 59.2% ± 8.8% at 5 years) (P=0.023).

Summary/Conclusions: The present study demonstrated that clinical and functional evidence for a collaborative association of RUNX1-MT and ASXL1-MT for sAML transformation. We identified HIF-1α targeting a new pathway which may be critical for leukemic progression of RUNX1/ASXL1-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 cytosine 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 (CMLL), 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 patterns 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 intra-cellular 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=6) had significantly lower DAC incorporation than non-responders (n=2), while other non-responders (n=2) showed low or no DAC incorporation (Fig E). Our method also enabled us to directly prove that low DAC incorporation was not a result of insufficient AZA accumulation intracellularly, 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.

Figure 1.

Summary/Conclusions: We have developed a new method that has enabled the first comprehensive analysis of the intracellular pharmacokinetics of AZA therapy in vivo. Our results have revealed that while AZA responders incorporated AZA efficiently into DNA, leading to DNA demethylation, there were two modes of primary AZA resistance: in some non-responders, low levels of AZA incorporation into DNA likely derives from cell cycle quiescence, resulting in low amounts of DNA demethylation. However, in other non-responders who showed DAC incorporation into DNA and demethylation, resistance arises from as-yet-unknown mechanisms not connected with AZA metabolism.

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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: To analyze the relationship between the dynamics of gene mutations and cell pathways they are involved in with the progression from MDS to sAML in order to study the mechanisms underlying disease evolution.

Methods: Sixty-eight serially collected samples from 34 MDS/CMLL 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/CMLL stage) and after sAML progression (disease evolution, leukemic phase). At initial presentation of the disease, diagnoses were as follows: 18 RAEB-1/2, 9 RCMD and 7 CMML. Initially, WES was carried out on 40 diagnosis/progression-matched samples. Driver mutations were identified, after variant calling by a standardized bioinformatics pipeline, by using the novel tool “Cancer Genome Interpreter” (https://www.cancergenomeinterpreter.org). Secondly, in order to validate mutations and precise variant allele frequencies (VAFs) estimation, TDS using a custom MDS/sAML-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, and only 25 occurring during progression. The most recurrently mutated genes were SF3B1 (21%), TET2 (21%), STAG2 (28%), SRSF2 (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 and clonal architecture. In the disease evolution dataset, we compared VAFs of mutations detected at both time-points (sAML to MDS/CMLL stage) in each patient. We identified 4 different clonal dynamics: mutations that were initially present but increased VAF (type-1), decreased (type-2), were newly acquired (type-3) or persisted with similar allelic burden (type-4) at sAML stage. Interestingly, most of type-1 mutations were detected in STAG2 gene. Thus, mutational burden of STAG2 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.
PRECLINICAL MODELING OF MYELODYSPLASTIC SYNDROMES
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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 pre-clinical model. However, the low level of engraftment of most of the patients is a limitation in the study of MDS. Here we have demonstrated the value of the PDX model 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 MDS prior to treatment of MDS patients.

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 benefiting 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 couple CD34+ cells, which again progressed to AML, mutations were lost or gained, associated with outgrowth of a clonal 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 limitation 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 MDS prior to treatment of MDS patients.

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PRECLINICAL MODELING OF MYELODYSPLASTIC SYNDROMES
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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 pre-clinical opportunity to capture the complexities of this myeloid malignancy. A number of PDX models have been developed, but the more promising to date are the NSG and the NSG-S (humanized with SCF, GM-CSF and IL-3). 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-SOGM 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 study clearly demonstrated that mitochondrial function is altered in MDS, leading to a strong energetic defect and an increase in oxidative stress, far beyond the expected parapathophysiological decrease resulting from ageing. The OXPHOS efficiency is highly reduced in MDS compared to controls, determining an impairment of the ATP/AMP ratio, which is 2.4 in young controls, 0.75 in elderly controls and it is 0.2 in i-thalassemia and MDS patients. By contrast, LDH activity increased in the MDS patients (6mU/mg) with respect...
the controls (88 μM/L), suggesting an attempt to compensate the energy imbalance with the increment of anaerobic glycolysis. MDA level, which reflects the lipid peroxidation, is 1 mM in young subjects, 9 mM in elderly subjects, 9 mM in b-thalassemia and 15 mM in MDS. In vitro iron chelation partially restored these abnormalities in MDS patients: ATP/AMP ratio increases from 0.2 to 0.6 in MDS and b-thalassemia, by contrast it is reduced in healthy subjects from 2.4 to 1.6. Anaerobic glycolysis is reduced after DFX incubation, in fact LDH decrease from 88 to 77 in MDS. By contrast, in healthy samples the iron chelation determined a reduction of OXPHOS activity, with a consequent impairment of ATP/AMP ratio and an increment of anaerobic glycolysis flux. Lipid peroxidation is significantly reduced by DFX and 23% with DFO (p value <0.01 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 normals. Findings suggest different treatment of patients with DFX reproduces similar findings as in vitro incubation.

Summary/Conclusions: In summary OXPHOS activity and the energetic status are highly impaired in MDS compared to elderly subjects. MDS cells used O2 to produce ROS instead of ATP. This is typical of ageing but is significantly increased in MDS compared to elderly controls and it is further increased by IOL. DFX is able to restore mitochondrial activity and ATP production in all the patients analyzed after in vivo or in vitro treatment.

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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 (CMML, 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 stimulants HS-5 cells, its culture supernatants (HS-5 sup.), immunomodulatory drugs (lenalidomide [LEN], pomalidomide [POM]), and anti-MDS agents (cytarabine and azacitidine), VSIG4 expression on cells was determined using FCM. 3) The proliferative potential was examined by BrdU incorporation using FCM and the MTT assay. 4) The cytotoxic activity of normal killer (NK) cell line NK-92-MIagainst target cells was determined by measurement of lactate dehydrogenase activity in culture supernatants. The production of IFN-γ from donor T cells was measured in the cell culture supernatants using ELISA.

Results: 1) VSIG4 mRNA expression in PBMCs from patients with AL-MDS was significantly higher than in those from controls. Cell-surface VSIG4 expression on CD14+CD11b+ monocytes from MDS and AL-MDS patients was higher than in those from controls, but VSIG4 expression was not detected on CD34+ blasts. In CD14+CD11b+ monocytes of MDS and AL-MDS patients, VSIG4 was strongly expressed on CD68+CD206+ tumor-associated macrophages (TAMs). Furthermore, the expression levels of VSIG4 on CD14+ mononoblasts from CMML patients were significantly upregulated in comparison with those from controls. 2) Two MDS cell lines expressed both VSIG4 mRNA and its cell-surface protein. VSIG4 expression on MDS cell lines, and on monocytes and monoblasts from MDS and CMML patients, respectively, was significantly upregulated by co-cultivation with HS-5 sup, LEN, and POM, but not with cytarabine or azacitidine. 3) VSIG4 expression on monocytes and monoblasts from patients with upregulated VSIG4 expression on target cells. CD45-mediated ADCC activity of NK92-MI cells against LEN-treated MDS cells was inhibited in comparison with untreated cells. Moreover, the production of IFN-γ from donor T cells co-cultivated with LEN-treated MDS cells was suppressed compared with control cells.

Summary/Conclusions: Our study demonstrated that VSIG4 was highly expressed on monocytes including TAMs in MDS and AL-MDS patients, and on monoblasts in CMML patients. VSIG4-expressing monocytes and monoblasts may suppress antitumor immune responses and be associated with disease progression in MDS and CMML. The results will allow us to elucidate the function of VSIG4 in MDS pathophysiology and lead to the development of new immunotherapy.

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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 interference of NEIL1 and OGG1, 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 (CMML) samples to identify neoplasia-specific targets for a synthetic lethality therapeutic approach. To validate the candidates through a direct strategy in an extended cohort of CMML, myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) patients.

Methods: We performed enhanced RNA-seq on 27 CMML bone marrow samples collected at diagnosis and 47 AML bone marrow samples collected at diagnosis. We generated consensus data to identify transcripts at a higher frequency in MDS and AML cases. We validated the microarray data by RT-PCR in an extension cohort of 73 additional CMML 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 oncogenes found to be overexpressed in our cmml patients or allowing for the inhibition of an specific DNA repair pathway (i.e. XPA, XRCC4, MSH4); ii) oncogenes infra-expressed in our cohort but with inhibitory molecules already being tested in myeloid neoplasms (i.e. PARP1). Global pattern of DNA repair gene expression was compared with MDS and AML MILE study data.

Results: Of 27 CMML patients and 10 healthy donors, the expression of 18 genes was significantly different between the two groups (p-value<0.05) with 6 genes up-regulated and 12 genes down-regulated in CMML patients compared with donors. Defects on genes predominantly unique to a single subtype were found. A few genes showed a significant difference in gene expression amongst AML subtypes. A total of 9 DEGs were identified in our AML subset. To validate this, we extracted the DNA repair transcriptional components from a large dataset of 206 MDS, 47 complex karyotype AML (AMLx) and 73 healthy donors bone marrow from the MILE study. Surprisingly, MDS misregulation was characterized for a predominance of upregulated genes (14 out of the 20 misregulated targets) while AMLx showed a global defect with a predominance of downregulation (37 out of 50 misregulated targets). Of note, some genes showed opposite sense of misregulation according to the myeloid disorder: TOP2P1 was upregulated in CMML cases but downregulated in MDS cases and viceversa for BAP1. CDK1 and EXO1 were upregulated in MDS cases and the opposite effect was found among AML cases.

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 CMML. In addition, the different sense of misregulation of these and other targets within the myeloid diseases, some of them already being targeted in the clinical trial setting, emphasize the need of a neoplasia-personalized test of DNA repair modulators.

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DIFFERENTIAL DIAGNOSIS BETWEEN MYELODYSPLASTIC SYNDROMES AND NON-CLONAL CYTOPEXIAS 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 of several origins (immune disease, hypersplenism, drug toxicity) were analysed by FC. We elaborated a Myeloid Maturation Database using the Infinicyt® v1.7 software (Cytognos, Spain). From all bone marrow controls, we merged files stained with a 4-colour combination (CD16-FITC/CD13-PE/CD45pC/CD11b-APC). We selected myeloid population from the merged file and drew a maturation path. We obtained a maturation diagram that displays the fluorescence intensity of each parameter measured along the maturation stages. Then, for patients and controls, we obtained the fluorescence intensities whose median values exceeded ±2SD range in comparison with the stored database values (Figure 1). We elaborated a score, considering the relevant changes in fluorescence intensities (deviations) in the four markers analysed (CD16, CD13, CD45, CD11b) and in the four maturation stages, with a punctuation from 0 to 16.

**Results:** We found a mean of 1.9 deviations (fluorescence intensities values exceeded ±2SD) in controls, and a mean of 4.5 deviations in patients. Our test resulted reliable for differential diagnosis between controls and patients (curve ROC analysis, AUC=0.746; p<0.016). We found that with a cut-off of 4.5 deviations, we obtained a high specificity in the diagnosis of MDS (100%) but a low sensitivity (45%). With a high suspicion of MDS (specificity 90%), we can consider patients with scores above 3.5, thus achieving higher sensitivity (59%). Additionally, the number of immunophenotyping changes correlated well with prognostic risk. We confirmed that the higher the 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 2).

**Summary/Conclusions:** The maturation database (using the maturation analysis from Infinicyt® software) was useful to discriminate between MDS patients and non-clonal cytopenias, proving to be a reliable diagnostic test, also with prognostic implications. The application of this database as a diagnostic tool has the advantage that the result is independent of the observer. Inclusion of more myeloid markers and incorporation of erythroid parameters could increase sensibility in differential diagnosis.
Background: Epigenetic drugs are currently used for the treatment of several hematologic malignancies, but their pharmacological mechanism remains poorly understood. For DNA methyltransferase and histone deacetylase inhibitors (DNMTi and HDACi) several mechanisms of action have been proposed, mostly based on candidate gene approaches. However, less 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 regulated 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 performed to characterize the epigenetic efficacy of multiple deacetylase inhibitors described by using a combinatorial single 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 transcription start sites (treatment-induced 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 immunogenetic 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-induced LTR12c activation. In agreement, HDACi specifically induced a subset of TINATS in association with H2AK8ac, 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 9-family members, epigenetically represented virtually all normal cells. Moreover, we found a consensus GATA2 binding motif which strongly distinguished LTR12c with active transcription start sites (treatment-induced non-annotated transcripts, TINATS). 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. Over-expression 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 chemotherapy 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 combined with cell cytotoxicity assays to further shed light on the interaction between epigenetic and immune therapy and the role of ERV-derived antigen presentation.

P665 IMPACT OF MARROW COMPLETE RESPONSE IN THE NATURAL HISTORY OF PATIENTS WITH MYELODYSPLASTIC SYNDROMES (MDS) AND CHRONIC MYELOMONOCYTIC LEUKEMIA (CMLM) TREATED WITH HYPMETHYLATING AGENTS

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Background: The concept of marrow complete response (mCR) was included in the response criteria remains controversial as it is not known how it affects the natural history of MDS, CML-M and CML-CP.

Aims: The aim of our study was to describe the impact of mCR in survival outcomes in patients with MDS treated with hypomethylating agents (HMA).

Methods: We retrospectively reviewed 619 patients diagnosed with MDS or CML-M and treated with frontline HMA between 2004 and 2015 at a single institution. Clinical and demographic data were obtained from an electronic data base. Response was assessed by modified 2006 IWG criteria. Statistical analyses were performed with the IBM SPSS Statistics 23.0 software. All tests were 2-sided with significance set at p<0.05.

Results: 444 (62.3%) patients from the initial cohort achieved at least hematologic improvement (HI) as best response and were included in the analysis. 162 (37%) patients were female. Median age at diagnosis was 68 years (range 17-91). Following the 2016 WHO classification: 30 patients (7%) were MDS-SD, 50 (11%) MDS-MLD, 20 (5%) MDS-RR, 230 (52%) MDS-EB, 10 (2%) MDS-U and 104 (23%) CML-M. According to the Revised International Prognostic Scoring System (IPSS-R), 37 patients (8%) belonged to the low risk group, 176 (40%) to the intermediate-1 risk group, 198 (45%) to the intermediate-2 risk group, and 31 (7%) to the high risk group. 200 (45%) patients received azacitidine-based therapies and 244 (55%) decitabine-based therapies. Responses included: 238 (33% of the total population) complete responses (CR), 61 (9%) mCR, 2 (<1%) partial responses (PR) and 143 (20%) stable disease (SD). HI was observed in 410 (58% of the total population) of the patients. The median time to response was 3 cycles (range 1-24). Median overall survival (OS) since the
LUSPATERCEPT INCREASES HEMOGLOBIN AND REDUCES TRANSFUSION BURDEN IN PATIENTS WITH LOWER-RISK MYELODYSPLASTIC SYNDROMES (MDS): LONG-TERM RESULTS FROM PHASE 2 PACE-MDS STUDY


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, 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 (Suragani HR 0.665 [95%CI=0.482-0.918]) (Fig. 1B). 

Methods: Inclusion criteria: MDS IPSS low or int-1, age ≥18 yr, Hgb <10 g/dL (if <4U RBC/8 weeks), no prior HMA, and no current lenalidomide or erythropoiesis-stimulating agent (ESA). The dose-escalation phase of the study is completed. An expansion cohort of up to 56 patients was added to this phase to allow adequate response evaluation. Luspatercept in pts who would not qualify for the phase 3 MEDALIST trial (for regularly transfused ring-sideroblast positive [RS(+)] patients with EPO <200 U/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 allowed if ≥1U RBC/8 weeks. Pts are treated every 3 weeks subcutaneously for up to 5 doses (titration up to 1.75mg/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 78% (5/7) for RS(+) pts with EPO 200-500 U/L. LTB-RIC rates for pts treated with ≥0.75mg/kg in the base and ext studies, respectively, were 68% (13/19) and 71% (10/14) for RS(+) pts vs 5% (1/22) and 2% (1/58) for RS(-) pts with EPO <200 U/L and 4% (1/23) and 5% (1/16) for RS(-) pts with EPO 200-500 U/L. Preliminary RS(-) response rates (IgW Hb-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 adverse events (≥2 pts) were diarrhea, fatigue, headache, hypertension, arthralgia, bone pain, injection site erythema, myalgia, and peripheral edema.

Results: Data (as of 09Sept2016) were available for 73 base and 42 ext study pts. Median and 22 ext pts were LTB/41 base ext pts had moderate 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 LTb pts was 8.6 (6.4-10.1). Median (range) RBC transfusion burden (U/8 weeks) for HTB pts was 6 (4-18). 71% base and 86% ext pts were RS(+). IgW Hb-E response 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 78% (5/7) for RS(+) pts with EPO 200-500 U/L. LTB-RIC rates for pts treated with ≥0.75mg/kg in the base and ext studies, respectively, were 68% (13/19) and 71% (10/14) for RS(+) pts vs 5% (1/22) and 2% (1/58) for RS(-) pts with EPO <200 U/L and 4% (1/23) and 5% (1/16) for RS(-) pts with EPO 200-500 U/L. Preliminary RS(-) response rates (IgW Hb-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 adverse events (≥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-TB. A Phase 3 study of luspatercept in regularly-transfused RS(+) patients with lower-risk MDS according to IPSS-R is ongoing (MEDALIST study; NCT02631070).

Figure 1.

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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: 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 MDS patients were allowed. Clinical response, cause of discontinuation, salvage treatments and OS from discontinuation of AZA were the major end points.

Results: Between January 2009 to June 2014 1799 newly diagnosed MDS patients were enrolled in the Registry, and 418 received AZA; 269 as 1st line treatment (64%), 112 as 2nd line treatment (28%), and 34 as a line ≥3rd (8%). Median age was 73 years (range 18-91); 260 patients (62%) were male. WHO diagnosis was RA or RARS (n=27, 6%), RCMD with or without RS (n=62, 15%), APEX (n=126, 30%), APEX 2 (n=189, 45%), other subtypes (n=15, 4%). At start of AZA therapy IPSS score was low in 14 (3.4%), int-1 in 97 (23.2%), int-2 in 163 (43.8%), high in 67 patients (16%), and not available in 57 patients (13.6%). Patients received a median of 7 courses of treatment (range 1-63). Seventy-three % of the whole cohort (418 pts) were alive at 1 year from beginning of AZA therapy and median OS was 23 months, (25 for IPSS lower-risk MDS and 21 for IPSS higher risk MDS). OS after discontinuation of AZA was 8 months. Clinical responses according to IWG criteria were available in 344/418 patients (82%). Of these, 152 (44%) achieved a complete hematological response, 77 (22%), a partial response, 86 (25%) had stable disease while 39 (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 11 (31%) patients, progression or loss of response and only in 10% of cases for reported toxicity. Only 28% of patients received any kind of salvage therapy and overall survival after AZA discontinuation was poor (8 months).

Methods: Children (0–18yrs) were referred from 14 UK centres with a diagnosis of suspected MDS and/or sustained cytopenias with morphological features of myelodysplasia. Extensive phenotypic information including family history, detailed clinical examination and disease course details were collected and captured on an online database using the Human Phenomiser tool. A customised targeted NGS panel was designed using the Illumina design studio containing 32 genes, 916 amplicons and 301 exons; selected through literature reports and well described mutations in Paediatric MDS and potential overlap Bone Marrow failure syndromes (BMFS). Coverage of each base within target regions was assessed for every sample on each sequencing run using Covemi software. Library preparation was performed using an Illumina TrueSeq Custom Amplicon panel, followed by sequencing on an Illumina MiSeq. Data analysis was performed using our established bioinformatic pipelines (Hamblin A: Blood 2014 124:2373).

Results: In total 59 patients (females= 29, males 30) have been screened and 3 subgroups identified based on the original suspected clinician diagnosis at presentation: MPN/JMML (n = 15), de novo MDS (n=9) and idiopathic cytopenias of undetermined significance, (ICUS) with some features of dysplasia (n=35). Mutations were detected in 24/59 patients (40%, Table 1). Of these, NGS results confirmed the original clinical diagnosis in 15 cases (62.5%); established the diagnosis for the first time in 6 cases (25%); and led to a change in diagnosis (from autoimmune neutropenia to Shwachman-Diamond Syndrome) in 1 case leading to a significant change in patient management. In two already known cases, it allowed monitoring of the disease molecular signature. As expected, PAS pathway mutations were common in the JMML/MPN (100%) and de novo MDS patient subgroups (33%). Additional mutations in epigenetic modifiers, spliceosome mutations as well as second PAS pathway hits were also detected in 40% of JMML patients and in one case within the de novo MDS group; this finding was associated with poor outcome. Within the heterogenous ICUS patient group, pathogenic mutations were identified in 5/35 (14.3%) cases with BMFS genes (SBDS, ELANE, TP53). In contrast to the other MDS/MPN cases, in this group, no PAS pathway mutations were detected.

Table 1.

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COMBINATION OF DEEP PHENOTYPING AND TARGETED NEXT GENERATION SEQUENCING AS A DIAGNOSTIC TOOL IN CHILDREN WITH SUSPECTED MDS

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Background: Paediatric Myelodysplastic Syndromes (MDS) are a rare and heterogeneous group of disorders distinct from adult MDS. They may present with syndromic anaemia, life threatening infection or evolving leukaemia; however, they may also present as unexplained cytopenias or with multisystem disease of unclear aetiology. Diagnosis can represent a huge challenge for clinicians, even in highly specialised centres and this can delay the delivery of the most appropriate treatment. Hence an accurate diagnosis is crucial in selecting the most appropriate surveillance and follow up strategy.

Aims: To devise a clinical grade diagnostic targeted NGS panel and combine the results with extensive clinical phenotypic information to obtain a diagnosis in children referred with suspected MDS.

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 patients 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.
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 DEEP RESPONSES IN MM POST-PBSCH (NCT01790737) 1.

Aims: This is the first study to report outcomes of patients treated with lenalidomide (LEN) maintenance therapy in the context of minimal residual disease (MRD) assessment. The aim of this study was to assess the proportion of different lymphocyte subsets at baseline and during LEN maintenance treatment.

Methods: Flow cytometry (FC) panel included antibodies against CD38, CD138, CD45, CD19, CD56, CD27, CD28, CD81, CD117, intracytoplasmic CD38+, CD19+, CD56+, CD27+, CD28+, CD81+, CD117+, and FC analysis was performed on bone marrow aspirates at baseline and during LEN treatment.

Results: Between April 2011 and January 2014, 153 patients were enrolled. Median age at registration was 58 years (range 26-79). The proportion of mature B cells in BM was significantly higher in the best response cohort at baseline. In particular, the median percentage of total lymphocytes (15.4 vs 11.9), CD38+ T-cells (0.9 vs 0.56), and CD38+ lymphocytes other than B-cells and NK-cells (0.9 vs 0.5) were all higher in the good response cohort at baseline. In particular, the median proportion of mature B cells in BM was significantly higher in the best cohort at baseline (1.32 vs 0.91; p=0.02), after induction (0.27 vs 0.13; p=0.002) and 16 months after ASCT (1.73 vs 0.56; p=0.008) (Figure 1).

Summary/Conclusions: Composition of the BM lymphocyte pool at treatment baseline may have an influence on treatment outcome in multiple myeloma. More detailed subtyping of the lymphocyte phenotypes is ongoing and may reveal potential predictive biomarkers for immunomodulatory drugs such as lenalidomide and checkpoint inhibitors.

Figure 1.
SUMMARY/CONCLUSIONS: Our study showed that PET-CT and MRI would represent the techniques of choice in the assessment of bone involvement in MM patients in view of their high and comparable sensitivity. Moreover, PET-CT allowed detecting skeletal involvement in 18% of cases negative by WBX, in 37% of those CT-negative, and in 10% of those MRI-negative. Sensitivity of PET-CT was superimposable to that of MRI (90%), and higher than that of WBX (60%) and CT (73%). Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS.


table 1.

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INITIAL PHASE 2 RESULTS OF IBRUTINIB COMBINED WITH Bortezomib/Dexamethasone IN PREVIOUSLY TREATED PATIENTS WITH PROGRESSIVE MULTIPLE MYELOMA

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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 AOU. Pisa, Italy, between January 1996 and December 2015. Eighty-three were male and 77 female; the median age was 70 years (range, 28-85), and half of them presented with low ISS risk score. Forty-five subjects were not eligible to high-dose therapy; 64% of them received bortezomib- and 23% melphalan-based regimens. Patients eligible to high-dose therapy received VAD, TAD or VTD and then one (88%) or two (12%) autologous transplants. At the relapse, lenalidomide (50%) or anthracyclines (40%) were administered. 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 group; p=0.016). Nevertheless, PET-CT positivity either at diagnosis or during follow-up did not impact on long-term OS and PFS.

Summary/Conclusions: 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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PROGNOSTIC SIGNIFICANCE OF CLONAL CIRCULATING PLASMA CELLS BY MULTI-PARAMETRIC FLOW CYTOMETRY IN PATIENTS WITH LIGHT CHAIN AMYLOIDOSIS UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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BACKGROUND: Presence of circulating plasma cells (cPCs) prior to autologous stem cell transplant (ASCT) is an adverse prognostic factor in patients with light chain amyloidosis (AL). cPCs are detected by multi-parametric flow cytometry (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 fLC, 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 fLC, BMPC, and Mayo Stage (data not shown).

In Group 2, both progression free survival (PFS) (10.5 months vs 58 months, p <0.0001) and overall survival (OS) (16 months vs not reached, p <0.0001) were worse in patients who had detectable cPCs compared to those without cPCs (Figure 1). This difference was not seen in Group 1 (OS: not reached vs 98 months, p=0.96; PFS 43 vs 52 months, p=0.74). In multivariate analysis, adjusting for Mayo Stage and induction chemotherapy, there was a trend towards worse OS in patients with detectable cPCs (p=0.06).

Table 1. Patients receiving induction chemotherapy before ASCT

<table>
<thead>
<tr>
<th>Group</th>
<th>Detection of cPCs</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Undetectable cPCs</td>
<td>0.6</td>
</tr>
<tr>
<td>Group 2</td>
<td>Detectable cPCs</td>
<td>&lt;0.0001</td>
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</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 prognostic 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 years 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 (p=0.06), 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, del1p), high LDH and high LDH were more prevalent in RI vs 46%, 30% vs 25%, respectively (p=0.01). Children were less common in advanced stage (ISS III) (66% vs 12% p=0.001). Anemia was more prevalent in RI (44% vs 14%, p<0.001), but bone lesions were less prevalent (52% vs 65% p=0.001). There was no difference in ECOG performance status. Most patients (87%) received Bortezomib-based therapy in first line (81% RI vs 91% no RI, p<0.001), with no difference with or without ASCT. Response rates (≥PR) were the same in patients with eGFR <60ml/min compared with normal renal function (84% vs 85%, p=0.87). PFS & OS decreased with reduction in eGFR (Fig 1). However, patients with eGFR15-30ml/min had better OS & PFS compared with eGFR 15-30ml/min; dialysis in eGFR <15ml/min may be a factor. Using age 70 yrs as a common age limit for ASCT, we analysed the effect of ASCT in patients <70 yrs with & without RI. While a smaller proportion of RI patients received ASCT (21% vs 79%, p=0.07), it was performed at all levels of renal function including eGFR <15ml/min. In patients with eGFR <60ml/min, those who received ASCT had a longer PFS (HR 0.37, 95%CI 0.16-0.88, p=0.03) & OS (HR 0.28, 95%CI 0.08-1.01, p=0.05) compared with no ASCT. The improvement was also seen in severe RI (<30ml/min), with a longer PFS (HR 0.21, 95%CI 0.05-0.86, p=0.03) & OS (HR 0.10, 95%CI 0.01-0.82, p=0.03) with ASCT.

Figure 1. Summary/Conclusions: RI occurred in one-third of newly diagnosed MM. DM, an underlying risk factor for CKD, was more common in RI patients, but not associated with a difference in outcome. Advanced stage & high risk features were more prevalent in RI patients, but bone disease was less common. RI patients had a shorter PFS and OS, overall correlating with eGFR. However patients with eGFR <15ml/min had a better OS than 15-30 ml/min, for which dialysis may be a factor. In transplant-eligible patients assessed by age <70 yrs, ASCT was performed in 21% of RI patients, at all levels of renal function. Patients with RI who underwent ASCT had a superior PFS and OS than those who did not have ASCT, including those with severe RI (eGFR <30ml/min), supporting the benefit of ASCT in MM patients with RI.

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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 and 30 (46%) pts had t(11;14). Median number of prior lines of therapy 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 1.8 (range: 0.1–15) months. Fifty-five (83%) pts discontinued, with 41 due to PD. Common adverse events (AEs) were nausea (47%), diarrhea (36%), vomiting (21%) and 23% of grade 3/4 hematologic toxicities [thrombocytopenia (23%), 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); 15% achieved very good partial response (VGPR) or beyond. Staging was updated to: SKY92 standard-risk 53 months, SKY92 high-risk 21 months. A clear difference in responses was seen among pts with t(11;14) vs without [ORR, 40% vs 6%; vGVPR, 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 were refractory to bortezomib, carfilzomib, lenalidomide, and pomalidomide, ORR were 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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**AN OPEN-LABEL, PHASE 1B STUDY (MMY1001) OF DARATUMUMAB COMBINED WITH CARFILZOMIB, LENALIDOMIDE, AND DEXAMETHASONE (KRd) IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) WITH t(11;14)**

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**Background:** The combination of daratumumab with standard of care regimens has demonstrated significantly prolonged progression-free survival (PFS), deeper responses, and a manageable safety profile versus standard care alone in patients with relapsed or refractory MM.

**Aims:** To determine the tolerability and efficacy of daratumumab in combination with KRd in patients with newly diagnosed MM.

**Methods:** This open-label, phase 1b study enrolled patients with newly diagnosed MM (t(11;14) confirmed). An open label, adaptive 3+3 dose escalation study was conducted in eight independent cohorts, at the time of its initial publication, and since.

**Results:** As of 20Oct2016, 22 pts were enrolled in the study; median (range) patient age was 60 (34–74) years, and 95% had an ECOG score of ≤1. At a median (range) follow-up duration of 7.4 (4.0–9.3) months, the majority of patients remained on treatment and had received a median of 8 (1–10) treatment cycles. Six patients discontinued treatment, 1 due to an adverse event (AE; pulmonary embolism), 1 due to progressive disease, and 4 due to “other” (all ASCT). The dose of carfilzomib was increased to 70mg/m2 in 19 patients by Cycle 1 Day 15. Serious treatment-emergent AEs (TEAEs) occurred in 46% of patients, 14% and 18% of which were at least possibly related to daratumumab or carfilzomib, respectively. Eighteen (82%) patients had a grade 3/4 TEAE, most commonly (>10%) lymphopenia (50%) and neutropenia (23%). One (5%) patient experienced a grade 3 cardiac TEAE (congestive heart failure) which resolved; the patient was able to resume study treatment at a reduced carfilzomib dose. There were no grade 5 TEAEs. Six (27%) patients had daratumumab-associated non-HSCT/SAEs, all Grade 1 or 2 in severity. ORR with daratumumab in combination with KRd was 100% among the 21 response-evaluable patients: 18 (86%) patients achieved a very good partial response or better and 1 patient (5%) achieved a complete response. Median duration of response has not been reached. With only 1 FFS event having occurred at the an of 1.4 months (range: 0.1–13). Fifty-five (83%) pts discontinued, with 41 due to lenalidomide, 35 (53%) to pomalidomide, and 52 (79%) were refractory to the last therapy, ORR was 42% (11/26); for t(11;14) pts refractory to both bortezomib and lenalidomide, ORR were 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:** Daratumumab in combination with KRd was well tolerated, with an overall safety profile consistent with previous reports for KRd. No additional toxicity occurred with the addition of daratumumab, and deep and durable responses were achieved. Preliminary data from this ongoing study support continued investigation of daratumumab plus KRd as a first-line treatment regimen for patients with newly diagnosed MM. Updated data based on longer follow-up will be presented.
Table 1. Multivariate survival analysis in the HO87/NM18 trial.

| 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), paralysis (HR=1.44; 95% CI 1.15-1.80), inflammatory bowel disease (HR=1.38; 95% CI 1.08-1.74), end stage renal disease (HR=1.57; 95% CI 1.03-2.04), and cirrhosis (HR=1.64; 95% CI 1.10-2.43).

**Summary/Conclusions:** Here, we compared the SKY92 classifier with revised ISS staging and FISH. These data validate the SKY92 classifier as a robust marker to identify high-risk patients in non-transplant eligible MM patients. In these IMiD treated patients, the SKY92, the revised ISS, and FISH markers such as deletion of 13q retain independent prognostic value.

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**MULTIPLE MYELOMA AND COMORBIDITY: A POPULATION-BASED STUDY**

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**Background:** The number of multiple myeloma patients has grown with aging populations, and with increasing age the number of comorbidities increases as well. Clinically, it is well known that comorbidity in multiple myeloma patients decreases performance status, increases risk of therapy-related complications and may lead to life-threatening conditions. Currently, the literature on comorbidity in multiple myeloma is very limited and based on small case series. Clinical trials rarely include elderly, frail patients due to eligibility criteria. Population-based studies provide valuable information on survival outcomes in relation to presence/absence of comorbidities in newly diagnosed real-life multiple myeloma patients in the general population.

**Aims:** To evaluate the prevalence of comorbidities and to study the impact of comorbidities on survival among patients with newly diagnosed multiple myeloma.

**Methods:** All newly diagnosed patients with multiple myeloma from January 1st, 1985 to December 31st, 2013 in Sweden were included in the study. Using the Swedish Patient Registry, all discharge diagnosis and discharge listings were gathered from each patient from January 1st, 1985. Comorbid conditions were defined as chronic illnesses which demand life-long treatment or follow-up. Only those diagnoses made prior to multiple myeloma were used. Using ICD 8.9 and 10 codes, comorbid diseases were identified. Kaplan-Meier curves were used to estimate survival. Risk of death was compared among multiple myeloma patients with a comorbid condition to those without a comorbidity, using Cox’s proportional hazards regression (adjusting for age, gender, year of diagnosis, and other comorbid conditions).

**Results:** 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), paralysis (HR=1.44; 95% CI 1.15-1.80), inflammatory bowel disease (HR=1.38; 95% CI 1.08-1.74), end stage renal disease (HR=1.57; 95% CI 1.03-2.04), and cirrhosis (HR=1.64; 95% CI 1.10-2.43).

**Summary/Conclusions:** 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.
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DETECTION OF NEW EMERGING CLONES DURING TREATMENT BY NGS ALLOWS A BETTER RISK PREDICTION ON MULTIPLE MYELOMA PATIENTS
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Background: Multiple myeloma (MM) is a genetically complex disease, characterized by the presence of multiple clones with differing degrees of drug sensitivity at the time of diagnosis. Consequently, therapeutic response of MM patients is unpredictable and extremely variable, and although the treatments introduced over the last decade have significantly improved the outcome of these patients, most patients eventually relapse. Deep sequencing methods have contributed to increase the knowledge about the clonal heterogeneity of the disease and helped to establish the three evolution patterns at relapse: linear and branching clonal evolution, and no clonal changes.

Aims: To analyze the diversity and relative dominance of different clones and their evolution throughout the course of disease by NGS of the immunoglobulin repertoire in MM patients. To evaluate if the presence of different clones is associated with increased risk.

Methods: Immunoglobulin repertoire was analyzed by NGS in bone marrow samples from 180 MM patients included in three GEM clinical trials (NCT00461747, NCT00443235 and NCT01237249). The two first clinical trials involve patients younger than 65 years old, and were analyzed with ChoroSeq methodology, the later one involve patients older than 65 years old, and were analyzed with a local NGS method recently validated (Martinez-Lopez et al, Laukemia 2017). A clonotype was identified when at least 40 identical sequencing reads were obtained, or it is present at a frequency of >1%.

Results: Of the 180 MM patients studied, 57 (32%) shows the presence of more than one clone throughout the clinical course of the disease. The identification of new evolving clones was only possible in the GEM10 clinical trial with the Local NGS method; in this clinical essay, 6% (4/71) of patients shows the development of different clones during treatment. We show that the frequency of the predominant clone at diagnosis of these four patients decreased with treatment, but the frequency of the new ones increased and the patients progressed. When more than one clone is present at diagnosis, the relative dominance of the clones varies throughout the course of disease in a fashion that is unpredictable in each patient. There were no differences in median MRD values between patients with one clone or more than one clone (0.0082% and 0.0055% respectively). The presence of more than one clone was not associated with high-risk cytogenetics. The presence of more than one clone at diagnosis does not condition the prognosis in any of the patients and treatments analyzed. Median PFS was 38 and 58 months for patients with one clone or more than one clone (0.0082% and 0.0055% respectively). Median age was 63 y, median time from diagnosis 5.1 y, median prior tx lines 2, and 94% had refractory disease. Cyto genetic data were available for 59 pts; 33% were high risk per IMWG. There were 9 dose-limiting toxicities, all asymptomatic cytopenias: 8 pts with grade (G) 3 lymphopenia and 1 with grade (G) 4 thrombocytopenia. The MRD was estimated at DL3. In 64 pts, G3/4 hematologic toxicities included neutropenia (25%) and lymphopenia (14%), and non-hematologic toxicities (all grades) included fatigue (51%), dyspnea (42%), and gastrointestinal (45%). PRs were high with a >PR rate of 63% after 1 cycle and 77% after 4 cycles. After a median of 20.9 cycles (range, 1–49), ≥PR rate was 95%, ≥vPR 77%, ≥CR 45%, ≥nCR 13%, and ≥ncomplete response (nCR) 20%. The 1st population (n=55, 51 LEN-refractory, 28 progressing on LEN maintenance), ≥PR was 84% with 30 treated at MTD. After median follow-up of 21 (1–49) mo, median progression-free survival (PFS) for all 64 pts enrolled was 16.8 mo and 2-y overall survival (OS) was 76.8% with 20 pts remaining on treatment. For standard-risk (n=38) vs high-risk pts (n=21), ≥PR was 89% vs 81%, ≥nCR was 24% vs 10% median, and OS was 22.3 ± 10.6 y, and 2-y OS was 90.8% vs 56.0%.

Summary/Conclusions: NGS is a well tolerated and highly active (>PR 84%) with encouraging PFS (median 16.8 mo) in a RRMM pt population that was mostly LEN-refractory and PI-naive/sensitive. The results support planned evaluation of NGS with daratumumab in RRMM, particularly for high-risk pts.

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PANOBINOSTAT INDUCES CD38 UPTAKE AND AUGMENTS THE ANTI-MYELOMA EFFICACY OF DARATUMUMAB
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Background: Immunotherapy with the anti-CD38 monoclonal antibody (mAb) daratumumab is increasingly being utilized in myeloma patients with relapsed/refractory (R/R) disease after prior treatment with immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs). However, the efficacy of dara tumumab is limited by low expression on neoplastic plasma cells. We inves tigate the use of the histone deacetylase inhibitor (HDACi) panobinostat to modulate target antigen expression on myeloma in favor of potent mAb-mediated recognition and destruction. We show that panobinostat augments CD38 expression specifically on myeloma cells and demonstrate powerful synergy with anti-CD38 mAb daratumumab.

Aims: Determine the impact of panobinostat on upregulation of CD38 expression on myeloma cells in order to enhance the efficacy of daratumumab.

Methods: Myeloma cells were treated with titrated doses of panobinostat (0, 10, 20, 25 nm) and expression of CD38 and a panel of additional target molecules including SLAMF7, as well as CD4+ T cells co-cultured with myeloma cells exposed to panobinostat at 24, 48 and 72 hours. Antibody-dependent cellular cytotoxicity (ADCC) against panobinostat treated and untreated myeloma cells was analyzed at 4 and 20 hours after addition of PBMC at an effector to target ratio of 25:1 in the presence of daratumumab or an isotype control antibody.

Results: Panobinostat treatment (n=12 patients) with panobinostat (10 vs 25 nm) and observed a uniform increase in CD38 expression in each case by flow cytometry. Upregulation of CD38 was already detectable after 24 hours, peaked after 48 hours of exposure to panobinostat and was higher at the 25 nm compared to the 10 nm dose. At 48 hours, the mean fluorescence intensity (MFI) of CD38 expression on CD138+ myeloma cells was increased 5.6-fold by panobinostat-treated compared to untreated myeloma (p<0.01). The increase in CD38 was equal in patients with previously untreated (n=5) and R/R myeloma (n=7); and could be confirmed in a panel of myeloma cell lines, including MM1.S and OPM-2. The panobinostat-induced upregulation of CD38 was rapidly reversible after drug withdrawal. Further, the effect of panobinostat on upregulation after panobinostat treatment was specific for myeloma and neither observed this phenomenon in a panel of leukemia and lymphoma cell lines, nor on primary CD8+ and CD4+ T cells that we isolated from peripheral blood of several donors.
and del(17p).

Responses in high vs achieving a VGPR or better. Median TTP (11.6 months) were longer for patients with high BCL2 expression (HRadj.=2.1 (95% CI 1.3–3.3), Fig. 1). In the period after the introduction of bortezomib 15% of patients became dialysis independent compared to 4% 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).

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 dependence, on renal function recovery.

Methods: All patients on renal replacement therapy (RRT) in the Netherlands are registered in the Dutch registry Renine. Data on age, gender, start date of RRT, type and switches of RRT or hospitals, primary renal diagnosis, date of death and cause of death are collected. In this nationwide population-based study, we selected all patients with MM registered in Renine between January 2002 and January 2016. No information regarding therapy of MM is provided in Renine. In March 2010, bortezomib was advised as first-line treatment in patients suffering from MM with renal impairment in the Dutch guidelines. Therefore, we divided our cohort in two periods: before the bortezomib guideline (January 1, 2002 till March 29, 2010) and after introduction of the bortezomib guideline (March 29, 2010 till January 1, 2016).

Results: A total of 700 patients were included in the study (422 patients pre-bortezomib and 278 after bortezomib introduction). In the period after the introduction of bortezomib 15% of patients became dialysis independent compared to 8% in the pre-bortezomib period (HRadj.=2.1 (95% CI 1.0–4.2) and HRadj.=5.7 (95% CI 2.5–13.2), respectively).

Figure 1.

Summary/Conclusions: In this nationwide population-based study, covering all patients with MM and concomitant renal failure, almost a two-fold increase of patients becoming dialysis independent occurred in the period after the introduction of bortezomib compared to the pre-bortezomib period. This was even more prominent when age was < 75 years and LCDD was the primary renal disease.
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: Aim of this study is to report the efficacy of pomalidomide and dexamethasone in patients with multiple myeloma-associated AL amyloidosis.

Methods: The databases of the Pavia Amyloid Research and Treatment Center were searched for patients with a diagnosis of multiple myeloma and AL amyloidosis. The final cohort included 61 patients who had received 28-day cycles of pomalidomide (4mg from day 1 to 21) and dexamethasone (20/40mg weekly). All patients gave written informed consent for their clinical data to be used for research purposes, in accordance with the Declaration of Helsinki. Thirty patients were treated to date. Hematologic and organ response were assessed according to the International Society of Amyloidosis criteria.

Results: Median age was 65 years (range: 34-85 years) and 22 (73%) patients were man. Heart involvement was present in 13 patients (43%) and kidney involvement in 18 (60%). Forty (13%) patients were in Mayo Stage I, 17 (57%) in stage II and 9 (30%) in stage III. Fifteen (50%) subject were in renal stage I, 8 (27%) and 3 (23%) were in renal stage II and III respectively and 5 (16%) patients were on dialysis at the time of P Dex initiation. Median bone marrow plasma cell infiltration was 20% (range: 12-90%). Twenty-three (76%) patients were refractory to all previous lines of therapy. Median time from diagnosis to treatment with pomalidomide was 71 months [interquartile range (IQR): 25-120 months]. Adverse events were observed in 5 (17%) of subjects: skin rash and confusion in one patient each and mild increase in serum creatinine in 3 (10%, resolved with the decrease of the dose of pomalidomide). The median number of prior treatments (excluding Alzheimer’s disease) was 3 (range 1-7). All patients previously received lenalidomide and an alkylating agent, only 3 patients were not exposed to bortezomib, due to severe peripheral nervous system involvement, 10 (33%) underwent autologous stem cell transplantation and 9 (30%) received previous thalidomide-based regimens. The median number of P Dex cycles performed was 4 (range: 1-11). Median follow-up of living patients was 6 months (IQR: 3.5-16 months) and 13 (43%) patients died due to progressive disease. Fourteen patients (47%) achieved at least partial response, with 1 complete remission (CR), and very good partial responses (VGPR) in 2 cases (6%). Cardiac responses were observed in 1 of 5 patients with measurable NT-proBNP (20%), but this can be underestimated due to the pomalidomide-related increase of NT-proBNP, and renal response in 3 of the 11 evaluable patients (27%).

Summary/Conclusions: The combination of pomalidomide and dexamethasone is well tolerated and effective in multiple myeloma-associated AL amyloidosis and can be a valuable rescue option in this high-risk population.

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WHEN PERFORMANCE OF CYTOGENETICS MATTERS: A POPULATION-BASED STUDY IN THE NETHERLANDS ON NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background: It was recently shown in both clinical and population-based series that unperformed cytogenetics (UPCs) in intensively treated patients with acute myeloid leukemia was independently associated with poor prognosis, as compared to patients with performed cytogenetics.

Aims: Therefore, we set out to assess whether UPCs is associated with poor outcome in young patients with symptomatic multiple myeloma (MM) who have received induction chemotherapy.

Methods: We identified 358 newly diagnosed patients with MM <66 years in one of the nationwide population-based Netherlands Cancer Registry (NCR). UPCs was used to indicate that no sample was sent in for cytogenetic analysis. Performed cytogenetics were grouped by Revised International Staging System (R-ISS), i.e. high-risk (presence of translocations (4;14) or (14;16) or deletion 17p) or standard-risk (presence of other aberrations or no aberrations). Only patients treated with induction chemotherapy, defined as treatment with VCD, PAD, BD or TAD +/- subsequent high dose dexamethasone and autologous stem cell transplantation (ASCT), were included for analyses. In total, 319 (89%), median age 60 years, 62% male were treated with induction chemotherapy, 39 patients (12%) had no therapy. The primary endpoint was progression-free survival (PFS), defined as time from start of first line induction chemotherapy to progression or death, whichever comes first. Patients alive without progression were censored at February 1st, 2016.

Results: In 230 (20%) patients, UPCs was used to indicate that no sample was sent in for cytogenetic analysis. Cytogenetics was performed and 63 of these patients (29%) were cytogenetically high-risk. No statistical significant differences were observed in CRB criteria or ISS between patients with or without performed cytogenetics. The proportion of patients undergoing ASCT was similar in different cytogenetic groups (standard-risk 85%, high-risk 83% and UPC 77%, p=0.77). Achieving partial response or better was higher, although not statistically significant, in the standard-risk group as compared to the high-risk and UPC groups (93% vs 89% vs 85%, p=0.92). Response outcome was unknown for 9% of the UPC group, 5% in high-risk group and 4% in the standard-risk group. Median follow-up time was 516 days. PFS for patients in the standard-risk group was highest, as compared to patients in the high-risk or UPC groups after one year of follow-up (88% vs 81% vs 74%, p=0.0003).

Summary/Conclusions: Our data show that cytogenetic testing is performed less frequently in the UPC group, which is 70% of MM patients <66 years. Although response rates were similar for patients in the UPC, standard- and high-risk groups, PFS was better in the standard-risk group. Patients with unperformed cytogenetics had the poorest outcomes. The reasons are unclear, but a plausible explanation for not performing cytogenetics could be the patients’ worse clinical condition at presentation. Therefore, UPCs requires immediate correction. As this is a cross-sectional study, no conclusions can be drawn. To our knowledge, this is the first study to address the correlation between UPCs and survival in MM patients treated with induction chemotherapy.

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 MYMY2002 (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 a proteasome inhibitor (PI) and an immunomodulatory drug (IMiD) (n=550, original 510, additional Swedish patients 40). Patients from the IMF-cohort who moved into further treatment lines after the line therapy where they fulfilled inclusion criteria, contributed information to the analysis for multiple lines of therapy, with baseline defined as the date of initiation of the actual treatment line, resulting in a total of 963 treatment lines from 550 patients treated with SOC. The relative survival 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 pretreatment frailty 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


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 due to early death in active MM.

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 GEM2010MAS65 (n=239) compared induction with sequential or alternating cycles of VMP + lenalidomide-dexamethasone. The event was defined as death related to active MM within 2 years from diagnosis, either because of disease progression or early death due to absence of response.

Table 1.

Results: From the 497 patients included, 77 (15%) patients died within 2 years from diagnosis due to active MM. When we compared this latter cohort with the remaining patients, the profile of the high risk group was characterized (Table 1) by a higher proportion of patients >75 years, advanced ISS and 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 CD227 MM phenotype. The multivariate analysis showed that the risk of early death due to active MM was related to four independent prognostic factors: age >75y (p=0.008), abnormal LDH (p=0.000), ISS 3 (p=0.008), and presence of HR CA (p=0.019). These four variables characterized by a high proportion of non-responding patients as well as unstaioned responses (umsR). The multivariate analysis showed that the risk of early death due to active MM was related to four independent prognostic factors: age >75y (p=0.008), abnormal LDH (p=0.000), ISS 3 (p=0.008), and presence of HR CA (p=0.019). These four variables characterized by a high proportion of non-responding patients as well as unstaioned responses (umsR).

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unsR (duration of response (≥PR) <6 months) to the baseline score we were able to build a new score in which the unsR had a 3 points weight. A score ≥ 4 identifies a subgroup of patients with high probability of death within 2 years despite optimized treatment.

Summary/Conclusions: The risk of early death due to active disease in elderly patients was related to four independent prognostic factors: age >75y, high LDH levels, advanced ISS, and presence of HR CA. A score ≥ 4 identifies a subgroup of patients with high probability of death within 2 years despite optimized treatment.

Background: Myelofibrosis (MF) may be caused by various pathogenic mechanisms, such as elevated circulating cytokine levels, cellular interactions, and genetic mutations. However, the underlying mechanism of MF remains unknown. A recent study showed that the neoplastic clone of fibrocytes, spindle-shaped fibroblast-like blood cells derived from monocyte lineage, was essential in primary MF pathogenesis; serum amyloid P, which suppressed fibrocyte differentiation, markedly improved survival and MF in a murine xenograft model (J Exp Med 2016; 213: 1723-1740). Regarding cytokines, the thrombopoietin (TPO) signaling pathway was assumed to be closely associated with promoting MF. Mice transplanted with TPO-overexpressing bone marrow cells showed symptoms such as MF and splenomegaly (Blood 1997; 90: 4369-4383). Romiplostim (Rom), a TPO-receptor agonist, induced MF in rats and some immune thrombocytopenic purpura patients (Blood 2005; 114: 3749-3756). Fibrocytes and TPO played certain roles in MF pathogenesis, but the nature of their relationship remains unknown.

Aims: We investigated the relationship between myeloproliferative leukemia protein (MPL; TPO receptor) activation and fibrocyte differentiation in promoting MF. The secondary goal was to discover a unique fibrocyte marker in monocyte or macrophage population.

Methods: Murine fibrocyte cell lines were established from transgenic mice harboring the temperature-sensitive large T-antigen gene of simian virus 40 under IL-13 and M-CSF conditions. Murine fibrocyte cell lines and human peripheral blood mononuclear cells (PBMCs) were cultured with or without Rom to evaluate if MPL activation promoted fibrocyte differentiation, and the ratio of spindle-shaped cells was calculated. Rom was administered on day 1 and 8 to induce an MF-like phenotype in C57BL/6J mice, and clodronate liposomes (CLs; day -4, -1, 4, and 7) were used to eliminate monocytes and macrophages.

Results: Flow cytometric analysis revealed that all murine fibrocyte cell lines stained positive for fibrocyte cell markers, including collagen I, CD45, CD34, CD11b, and CD68. Murine fibrocyte cell lines expressed MPL and responded to Rom or murine TPO to differentiate into mature fibrocytes, specifically with a longer spindle shape and stronger collagen I expression than when cultured with IL-13 and M-CSF alone. Rom also increased the number of mice spleen cell fibrocyte colonies in the presence of IL-13 and M-CSF. The administration of 1mg/kg of Rom once a week induced an MF-like phenotype in all mice within 2–3 weeks and increased the number of fibrocytes in the spleen. Treatment with CLs eliminated fibrocyte precursors and prevented severe MF and splenomegaly. Human cultured fibrocytes also expressed MPL, and Rom increased the number of spindle-shaped fibrocytes induced from human PBMCs. The SLAMF7high MPLhigh subpopulation was clearly separated from the SLAMF7low MPLlow population in human CD14+ monocytes. A significantly higher frequency of fibrocyte differentiation was observed in the SLAMF7high MPLhigh population. The number of SLAMF7high MPLhigh cells was significantly greater in MF patients than in healthy donors. Conversely, their numbers did not increase in MF patients treated with ruxolitinib.

Summary/Conclusions: MPL activation directly induced fibrocyte differentiation from monocytes and macrophages expressing MPL, and the elimination of these cells reversed the MF phenotype. Our findings confirmed a link between fibrocytes and the TPO/MPL signaling pathway and indicated that the combination of MPL and SLAMF7 could be a useful fibrocyte marker in monocytes or macrophages.

Background: Progressive bone marrow (BM) fibrosis in patients with PMF is thought to arise from non-hematopoietic stromal cells stimulated by overpro-
duced growth factors. However, in other tissues and organs, fibrosis is associated with the expression of fibroblast markers and stromal cells. Recently, we have reported that clonal neoplastic fibroblasts play a role in the induction of BM fibrosis in primary myelofibrosis (PMF) (Verstovsek, J Exp Med. 2016). We demonstrated that the BM of PMF patients harbors neoplastic, functionally distinct fibroblasts and fewer hematopoietic normal bone marrow. In addition, we detected an overabundance of fibroblasts in the BM and spleen of an established PMF mouse model and a xenograft mouse model of PMF created using BM-derived low-density cells from patients with PMF.

Aims: Fibroblasts, which make up <1% of BM cells, differentiate from a subpopulation of CD45+ monocytes and are recruited to sites of organ damage where they regulate tissue repair. We hypothesized that clonal neoplastic CD14+ monocytes may play a role in the induction of BM fibrosis in PMF.

Methods: To test this hypothesis, we transplanted NSG mice (NOD/SCid Il2rgtm1Wempl/Sjz) with sorted CD14+ monocytes from patients with JAK2V617F-positive PMF or donors with hematologically normal BM.

Results: Here, we show that BM-derived CD14+ cells from patients with JAK2V617F-positive PMF or donors with hematologically normal BM engrafted in NSG mice. Transplanted NSG mice with PMF BM-derived CD14+ monocytes developed a myelofibrosis-like phenotype with reticulin fibrosis and abundant neoplastic (JAK2V617F) fibroblasts in the BM and spleen. Two months after transplantation, we detected a subpopulation of hCD45+ and hCD68+ cells within the HLA+ population of BM cells. In addition, we found dysplastic megakaryocytes in the BM and spleen of the PMF CD14+ transplanted mice. Immunohistochemical staining of paraffin-embedded BM sections did not detect hCD3, hCD19 or hCD34 cells. However staining with anti-human CD42b antibodies detected human megakaryocytes, suggesting that the dysplastic megakaryocytes detected in PMF CD14+ transplanted NSG mice are human-derived.

Summary/Conclusions: Taken together, our data suggest that neoplastic CD14+ monocytes contribute to the induction of BM fibrosis in PMF. What role CD14-derived megakaryocytes play in the pathogenesis of PMF remains to be determined.
MOLM-13, MV4-11, KG-1 and U-937, the eosinophilic leukemia cell line EOL-1, human cultured umbilical vein endothelial cells (HUVEC), the microvascular human endothelial cell line HMVEC-1 and primary neoplastic cells obtained from patients with AML, chronic myelomonocytic leukemia (CMML) and (clonal or reactive) hypereosinophilia were used. Cell proliferation was quantified by 4H-thymidine uptake. Apoptosis was determined by flow cytometry and light microscopy. The phosphorylation-status of KIT and BTK was analyzed by Western blotting. The effects of DCC-2618 on histamine secretion in basophils (BA) were analyzed by histamine release assay.

Results: DCC-2618 was found to block the proliferation of all MC lines tested, with lower IC50 values measured in KIT D816V-negative HMC-1.1 cells (12±2.3 nM) and ROSA-KITWT cells (4±1.5 nM) than in KIT D816V-positive HMC-1.2 cells (123±36 nM), ROSA1T1BD186V cells (182±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 JAK2-activating mutation. For the ASAH1 and MCL (IC50: 83-460 nM). DCC-2618 induced apoptosis and blocked tyrosine phosphorylation of KIT in all cell 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±86 nM) and MOLM-13 (IC50 132±95 nM). In addition, DCC-2618 was found to block proliferation in primary leukemic cells in patients with monoblastic AML and CML 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-VEGF-induced histamine release from normal BA in a dose-dependent manner (IC50: 1-10 µM).

Summary/Conclusions: DCC-2618 is a new potent switch control TKI that counteracts growth and survival of neoplastic MC, leukemic monocytes, AML blasts, eosinophils, and endothelial cells in vitro. Whether DCC-2618 is able to block growth of neoplastic MC and other involved lineages in patients with advanced SM is currently being ascertained in a clinical trial (NCT02571036).

P694 DISTRIBUTION OF MUTATIONS IN DRIVER AND NON-DRIVER GENES ACCORDING TO CLONAL HEMATOPOIESIS IN ESSENTIAL THROMBOCYTHESMA, POLYCYTHEMIA VERA, TETRAOXYGENASE MOTHER (PV) 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 JAK2, 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 of patients at the time of HUMARA analysis was 64 years (range:21-92). Mutations in JAK2 were present in 62% of them, CALR in 11%, MPL in 8%, and 14% were triple negative (TN). Non-driver mutations were detected in 56 patients (17 PV and 28 ET). The most frequently mutated genes were TET2 (16%), DNMT3A (8%), ASXL1 (5%), SF3B1 (5%), EZH2 (2%) and RUNX1 (2%). The mutation with the highest variant allele frequency (VAF) was considered the dominant mutation and it corresponded to a driver mutation in 92 patients (JAK2 n=70, CALR n=13, MPL n=8) and a non-driver mutation in 9 patients (JAK2 n=2, CALR n=1, MPL n=1). In 9 patients, the JAK2 mutation carried the TET2 mutation (n=4) or the CALR mutation (n=5) or the MPL mutation (n=1). In 12 cases the VAF of the driver mutation (JAK2 n=9, CALR n=1, MPL n=2) were similar to the non-driver mutation, being TET2 the codominating mutation in 6 of them. HUMARA analysis was clonal in 66 patients and polyclonal in 60 patients. In 8% of patients, the driver mutation was most frequently observed in PV (76%) vs ET (44%; 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 than in TN women (n=43, 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). Age was significantly associated with clonal hematopoiesis and with the presence of non-driver mutations (median age of 55 and 68 years for polyclonal and clonal HUMARA respectively, p<0.0001 and 57 and 71 years for patients without and with additional mutations respectively, p=0.001). In the multivariate analysis, the variables that were associated with a higher probability of clonal hematopoiesis were the presence of non-driver mutations (OR:4.2, 95%CI: 1.6-10.6, p=0.003), age> 65 years (OR:2.7, 95%CI: 1-2.6-6.5, p=0.02), MPL genotype (OR:10.9, 95%CI: 1.2-98.9, p=0.0001) and PV diagnosis (OR:3.6, 95%CI: 1.3-9.7, p=0.009).

Summary/Conclusions: The presence of non-driver mutations is associated with clonal hematopoiesis regardless of age and type of disease. Elderly patients with clonal hematopoiesis often show non-driver mutations coexisting in equal or greater proportion than the driver mutation suggesting that MPN has originated on preexisting clonal hematopoiesis.

RUXOLITINIB/NILOTINIB/PREDNISOLONE COMBINATION: A PROMISING NOVEL TREATMENT FOR MYELOFIBROSIS

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Background: Myelofibrosis (MF) is the myeloproliferative neoplasm chromosome Ph-negative with worst prognosis. MF is characterized by stem cell-derived clonal myeloproliferation and reactive cytokine-driven inflammatory bone marrow fibrosis. Ruxolitinib is the first line treatment for MF. It was associated with significant reduction in symptomatic splenomegalgy and improved constitutional symptoms. In a previous work (Arenas et al. Blood Volume 122, Issue 21 (ASH Annual Meeting Abstract)) we identified a set of promising synergistic drugs combinations for a ruxolitinib. Nilotinib and prednisolone were selected from them.

Aims: The aim of this work is the study the effect of the combination of ruxolitinib, nilotinib and prednisolone in hematopoietic progenitor cells from patients with MF.

Methods: A ruxolitinib, nilotinib and prednisolone dose-response curves and synergistic studies were performed in hematopoietic progenitors CD34+ from five MF patients. We studied the molecular effect of single drugs and in combination on SET2 cell line with western blot. To adress the antifibrogenic activity of the drugs and their combinations, we pre-incubated HS27 cultures with 100nM of ruxolitinib, 1 µM of nilotinib, 1 µM 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).

Figure 1. Results: The effects of ruxolitinib, nilotinib and prednisolone resulted in an EC50 value of 55nM, 6.6µM and 13.1µM, respectively. A combination index (CI) of less than 1 indicated synergy. All combination had a synergistic behavior (Table 1); moreover, there were two combinations whose CI from all samples was less than 1: ruxolitinib/prednisolone (CI=0.8, p<0.05), nilotinib plus 0.8 µM prednisolone (CI=0.25±0.11) and 32µM ruxolitinib plus 0.8 µM prednisolone (CI=0.45±0.11). The JAK/STAT signaling pathway was inhibited: the phosphorylation of STAT5 was inhibited by ruxolitinib in 83.2±10.8 % (p<0.05) controlling to regard at 30 min and it was maintained at 3 hours (p<0.05). The combinations 32nM ruxolitinib plus 1.6 µM nilotinib (RN) and 32µM ruxolitinib, 1.6µM nilotinib plus 0.8µM prednisolone (RNP) inhibited more than 50% of the phosphorylation of STAT5 at 30 min and maintained at 3 hours. The MAPK signaling pathway was inhibited at 30 min, the phosphorylation of ERK was inhibited in 77±1.7%. 

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The coefficient of variation of 11.91%, and 26.04%, respectively. The Akt/Pi3K signaling pathway seemed to begin to inhibit 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 antibiogenic activity of the drugs and their combinations were studied. Nilotinib reduced the mRNA expression of collagen (21.9±11.9% (p<0.05) and prednisolone (RN: 37.8±1.9% (p<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 anti-fibrotic 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.

Table 1.

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<th>Sample</th>
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Summary/Conclusions: Gene mutation analysis by targeted NGS in myeloid malignancies is highly reproducible between laboratories and allows a comprehensive characterization of patients. However, some challenges are identified: indels, low frequency mutations (<10%), ASXL1TG464fs detection and variant categorization are critical points that have to be addressed to improve the results. Test system validation is crucial for the implementation of NGS technology.
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 #2007-005306-49). 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 Thrombocytopenia (ET) and 22 Polycythemia Vera (PV) patients (23 F/17 M) with a mean age of 62 years (range 29-81) were assessed. JAK2V617F was detected in 77.5% (n=31/40). Complete clinical response (CR) was achieved in 8 patients, partial (PR) in 17, and no response (NR) in 15 patients. MA was on average 8.3 years younger than CA (range -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 to -3.7) and after therapy (-13.0 years to -4.3). A statistically significant link between JAK2 allelle burden and MA was seen: compared to patients with low or no JAK2 allele burden, patients with high JAK2 (>60% at baseline) had an older MA at trial entry (64.2 years vs 44.5, p=0.0007) and after therapy (64.3 years vs 44.6, p=0.0015). This difference was also seen when PV or ET patients were examined separately. Patients with a high JAK2 allele burden tended to have a MA closer to their CA at trial entry (-0.8 years vs -15.3, p=0.0122) and after 3 months therapy (-0.5 years vs -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).

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 NEOPLASM INITIATION AND PROGRESSION
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Background: The number of detectable somatic mutations increase with age, but this increase is surpased by the rise in the incidence of cancer in older people. The underlying mechanisms for this disparity remain to be elucidated. Myeloproliferative neoplasm (MPN) is an ideal malignancy model disease to study clonal hematopoiesis, disease initiation and progression during natural aging because the majority of the relevant mutations (such as JAK2 V617F) are catalogued, the disease evolves and progresses slowly allowing the collection of serial samples, and an inducible transgenic mouse models for the disease have been established. Nonetheless, the prevalent occurrence of such clonal events in aged individuals brings up the question, which age-associated factors contribute to initiate hematologic malignancies and what are the rate limiting steps attributable for age-induced myeloid malignancies? We hypothesize that the reduced hematopoietic stem cells (HSCs) and their progeny provides a context that favors a greater number of new mutations, selection for pre-malignant clones, and that activation of mutant JAK2 further augments these changes for increased MPN incidence in aged individuals. Thus, delineation of age associated cellular and molecular mechanisms attributable for increased prevalence of myeloid malignancies will be essential for the development of strategies for early detection and therapeutic targeting of myeloid malignancies.

Aims: The goal of this proposal is to identify age associated hematopoietic cell-intrinsic and cell-extrinsic factors that determine initiation and progression of MPN at young versus old age in mouse models carrying a JAK2-V617F or JAK2V617F;+12 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. Integated omics analysis was performed on MPN initiating stem and progenitor cells.

Results: Our results suggest that age related changes in expression patterns respond to a set of factors that control cell fate decisions 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 common diseases, the 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.

Myeloproliferative neoplasms - Clinical 2
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%), splenomegaly MF (71% vs 57%), and high DIPSS score (41% vs 14%). For those with BL anemia regardless of whether RBC transfusion-dependent (TD), PAC did not worsen hgb levels and the rate of clinical improvement in hgb was higher for pts in the PAC BID arm (25%) vs PAC QD (13%) or BAT (12%) arms (Table). For the BID arm, CALR RBC transfusion independence (TI) at BL (TD 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 achieved RBC-Ti by week 24. In PAC pts, SVR >33% 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, 9% and 13 weeks 8-16 for QD and BAT, respectively) and first 8 weeks of BAT (10%). For pts with BL hgb <10 vs 10-11 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 QD (11% vs 12%) or BAT (20% vs 24%) (Table). In instances of high DIPSS, 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.

Objective response after 12 cycles (n=4), leukemic transformation (n=4) or death (n=13; 13 pts (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 interval (TFI) and 10 pts (26%) on treatment for 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. PAC treatment led to clinical improvement and the results from our MPNSG-0109 trial, a step-wise increase of the POM dosage is included for the 2nd study cohort to further improve anemia response.

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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 0.5-2mg POM once daily (QD) (Schlenk RF, Steigelmann 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: This multicenter, single-arm phase-IIb trial with a target population of 38 pts in the first cohort. Primary endpoints are response rate after 12 cycles (28 days each) according to IWG-MRT (Tefferi et al., 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 dose-modifications being allowed.

Results: Safety and efficacy data from 38 pts are presented. Median age of the 38 pts was 65 years (range 40-83); 19 pts (50%) previously received hydroxyurea, 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 CALR are 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 (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 occurring in 13 pts (34%); fatigue followed by fatigue in 12 (32%). Treatment interruptions were rare. There were 29 serious AE (SAE) CTCAE ≥2-5: 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, leukemic transformation, abdominal pain, septic shock, respectively). Five pts (13%) had prior RUX therapies. Two pts (5%) had prior exposure to cytotoxicity, “3” fatigue, “3” 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
Methods: Aims: This analysis includes 418 pts (primary MF, 66%) who were ≥75 y and started treatment ≥1 y before data cutoff (01 Jan 2016). Baseline characteristics (median) were age, 78 y (range, 75-89 y); male, 57%; spleen length, 20 cm (0-35 cm); blast count ≥1%, 30.3%; hemoglobin, 101 g/L (<100 g/L, 46.9%); PLT count, 249×10^9/L (<100×10^9/L, 6.3%). ECOG PS <2, 84.9%. At data cutoff, more than half of pts remained on treatment or completed treatment per protocol (52.6%). As expected, a greater proportion of elderly pts discontinued RUX due to adverse events (AEs; 23.6%) or death (8.7%) than pts in the overall study (17.7% and 4.1%, respectively). Overall, 72.4% of pts had dose modifications (AEs, 58.4%), and 33.9% had an interruption (AEs, 31.5%). Safety of RUX in elderly pts was consistent with that in the overall population. Median exposure was 11 mo; mean average daily dose was 26.8 mg (SD, 10.6). The most common hematologic grade 3/4 AEs were anemia (43.8%; overall, 34.1%) and thrombocytopenia (22.1%; overall, 16.3%). AEs (all grade [grade 3/4]) in >10% of pts included asthma (18.3% [2.6%]), pyrexia (18.0% [2.6%]), dyspnea (14.4% [4.3%]), diarrhea (13.5% [1.9%]), fatigue (11.8% [2.4%]), peripheral edema (10.8% [0.2%]), and pneumonia (10.1% [7.2%]). Infections in >5% of pts included pneumonia (10.1%), urinary tract infection (7.0%), and bronchitis (5.1%). Herpes zoster occurred in 3.9% of pts. At wk 24, 56.4% (124/220) of pts had a ≥50% reduction from baseline in spleen length (overall, 56.6%), and 19.1% (42/220) had 25%-50% reductions (overall, 23.3%); rates were similar at wk 48 (54.6% [65/119] and 19.3% [23/119]; overall, 61.6% and 18.9%). Most pts (64.2%) achieved a ≥50% reduction at any time (Figure 1), similar to the overall population (70.2%). Pts also experienced significant improvements in symptoms. From wk 4 to 48, 42%-48% and 50%-57% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACT-Fatigue, respectively.

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. Summary/Conclusions: In the phase 3 PERSIST-2 study of PAC vs BAT in pts with MF and BL thrombocytopenia, although 19% of RUX-treated pts achieved a 50% reduction in TSS, RUX-treated pts rarely achieved SVR ≥35% at week 24. Rates of grade 3/4 AEs were higher with PAC vs RUX treatment, though the majority of RUX-treated pts began with 5mg dosing. Rates of dose reductions and discontinuations due to AEs with PAC BID and RUX were similar. Following crossover to PAC in 22 RUX-treated pts, 19 remained on treatment at the time of data cut-off.

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SAFETY AND EFFICACY OF RUXOLITINIB (RUX) IN ELDERLY PATIENTS (>75 YEARS) WITH MYELOFIBROSIS (MF): AN ANALYSIS FROM THE PHASE 3B, EXPANDED-ACCESS JUMP STUDY

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Background: RUX is a potent JAK1/JAK2 inhibitor that has led to reductions in splenomegaly and symptoms in patients (pts) with MF. Although few studies have assessed RUX in elderly pts, a recent analysis including 100 pts ≥75 y showed that RUX was safe and effective in these pts, with safety and efficacy outcomes similar to those in younger pts (Lattaglia et al. Blood 2016;128:4251), JUMP, a large (N=2233), phase 3b, expanded-access trial assessed safety and efficacy of RUX in pts with no access to RUX outside a clinical trial and included a cohort of pts ≥75 y. Results: ed changes in spleen length and symptoms. From wk 4 to 48, 42%-48% and 50%-57% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACT-Fatigue, respectively.

The most common hematologic grade 3/4 AEs were anemia (43.8%; overall, 34.1%) and thrombocytopenia (22.1%; overall, 16.3%). AEs (all grade [grade 3/4]) in >10% of pts included asthma (18.3% [2.6%]), pyrexia (18.0% [2.6%]), dyspnea (14.4% [4.3%]), diarrhea (13.5% [1.9%]), fatigue (11.8% [2.4%]), peripheral edema (10.8% [0.2%]), and pneumonia (10.1% [7.2%]). Infections in >5% of pts included pneumonia (10.1%), urinary tract infection (7.0%), and bronchitis (5.1%). Herpes zoster occurred in 3.9% of pts. At wk 24, 56.4% (124/220) of pts had a ≥50% reduction from baseline in spleen length (overall, 56.6%), and 19.1% (42/220) had 25%-50% reductions (overall, 23.3%); rates were similar at wk 48 (54.6% [65/119] and 19.3% [23/119]; overall, 61.6% and 18.9%). Most pts (64.2%) achieved a ≥50% reduction at any time (Figure 1), similar to the overall population (70.2%). Pts also experienced significant improvements in symptoms. From wk 4 to 48, 42%-48% and 50%-57% of pts achieved a clinically meaningful response on the FACT-Lym TS and FACT-Fatigue, respectively.

Figure 1. Summary/Conclusions: This analysis included the largest cohort of elderly pts with MF treated with RUX to date. Consistent with the study by Latagliata et al. RUX was safe and effective in pts ≥75 y, with pts achieving reductions in splenomegaly and symptoms similar to those in the overall population, with comparable rates of AEs. Additionally, findings from our study were consistent with those of the COMFORT studies, which included few pts ≥75 y. Overall, our study provides further evidence that RUX is safe and effective in elderly pts with MF.

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PROGNOSTIC RISK MODELS FOR TRANSPLANT DECISION-MAKING IN MYELOFIBROSIS

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Background: Accurate disease risk stratification is crucial for transplant decision-making in myelofibrosis (MF), since 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 set of 544 MF patients aged 70 years or younger at time of diagnosis.

Methods: The Spanish Registry of Myelofibrosis is a nationwide, longitudinal registry contributed by centers associated to the Grupo Español de Enfermedades Mieloproliferativas Filadelfia 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 R² explained variation. All the statistical analyses were performed with IBM SPSS 22.0 and Stata 11.

Results: A total of 544 patients were included in the analysis. The median age was 62.6 years (IQ range: 54.9-69.7). There were 311 males and 233 females. The number of patients in the intermediate-2 and high risk categories was smaller in the DIPSS than in the IPSS or the DIPSS-plus. Overall, the Rumi’s score yielded the highest power to discriminate between risk categories, as measured by the C-index and the R² explained variation. However, the IPSS and DIPSS-plus were the best models to discriminate between the intermediate-1 and intermediate-2 risk categories, which is the critical cut-off point for patient selection to transplant.

Summary/Conclusions: In our contemporary series of MF patients only the high risk category of the current prognostication systems have a median survival below the 5-year threshold recommended for considering transplantation. Patient selection for transplant 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 thrombocythemia (ET), hydroxyurea (HC), has mutagenic properties and there is potential for leukemogenicity and secondary cancers with this agent. Although leukemia (AML) and other malignancies in HC-treated patients compared with those treated with anagrelide (ANA). However, there were large age differences between groups. Here, we account for age differences by estimating standard incidence ratios (SIRs) using country-specific cancer registration data.

Aims: To assess the risk of AML and non-hematological malignancies in patients treated with HC or ANA in the EXELS study.

Methods: Previous exposure to ANA and HC was based on patient history. SIRs were calculated using background rates retrieved from Cancer Incidence in Five Continents (CI5). Risk of AML after study enrolment was estimated by cumulative incidence. Minimum exposure time of 180 days was used to account for disease progression as a confounding factor. Informed consent was obtained before the start of the study.

Results: Overall, 3460 patients were exposed to HC, ANA or both at registration; 481 patients had ANA treatment, 2305 had HC treatment and 674 had been exposed to both drugs. The median age in ANA patients was 51 years, and 71 years in HC patients. One hundred and seventy four cases of non-hematologic cancer, including 35 cases of skin cancer, were recorded. SIRs for all malignancies were close to 1 for all treatment groups, indicating similar risks to the background population. For all skin cancers, including melanoma, the SIR for patients with HC treatment was higher than expected for the normal population and patients on ANA (1.15 vs 0.45). When melanoma was excluded, the figures changed only marginally. However, due to the low number of events, the CIs were wide, and no statistically significant difference was found between treatments. Sixty seven AML cases were found; 39 AML cases were found in the HC group (person-years 8970, SIR 39.7), with another 20 AML cases in the group who switched from HC to ANA (person-years 2934, SIR 91.5). The risk ratio for AML developing in patients who switched from HC more than doubled (RR 2.30-2.52), irrespective of minimum exposure time. In contrast, no AML case was observed in the ANA-only group (person-years 1905, SIR 0) and there were only 3 AML cases in the group who switched from ANA to HC (person-years 802, SIR 68.5). Since the number of AML cases in the ANA group was 0, no statistical comparison could be made. Five AML cases were excluded from analysis since there was uncertainty about which drug was used first.

Summary/Conclusions: ET patients have a substantially increased risk for AML development; the SIR for AML was 40-fold higher than expected with HC exposure. Patients who switched from HC to ANA the SIR was approximately 90-fold higher, with 20 AML cases observed versus the expected 0.22 cases. The number of AML cases was much lower (n=3) in patients who switched from ANA to HC. It has been proposed that exposure of ET patients failing HC therapy to a second potentially leukemogenic agent should be avoided; yet these data suggest that even after switching to a non-leukemogenic agent, HC-treated patients still have an over 90-fold increased risk of AML. Our data reinforces concerns of a leukemogenic effect of HC, with a markedly higher risk in patients failing HC and switching to another drug, even if that drug is ANA. The caution advocated in the use of HC seems well advised.
Background: Infectious complications represent one of the most frequent causes 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); Hb, 10.8g/dL (7-16.7); Hb <10g/dL, 40%; PLT, 246×10^9/L (33-1887); PLT <100×10^9/L, 10%; spleen enlargement, 97%; spleen length ≥10cm, 62%; 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=0.0001). Respiratory tract infections were more frequently observed (73 events, 57%). Cutaneous, urinary tract and gastrointestinal infectious events were diagnosed in 15%, 10% and 7% of cases, respectively. In 14 cases fever of unknown origin was recorded (Figure 1). Etiological agents were isolated in 14 cases (11%); bacteria in 9 cases (gram+ 56%, gram- 22%, C. difficile diarrhea 22%) and fungi in 2 cases (pulmonary aspergillosis and oesophageal candidiasis). Mycobacterium tuberculosis Mycosis was isolated in 3 cases. Herpes-virus reactivations occurred in 12 cases (9%). No patients reactivated hepatitis B virus. At last follow-up, 88 pts (24%) have died, in 10 cases (11%) due to infectious complication. Among baseline features, age≥65 years at RUX start (p=0.0001), previous infection (p=0.001), primary vs secondary MF (p=0.021) and high IPSS (p=0.029) significantly correlated with higher infectious risk. Notably, no differences were observed according presence of large (≥10cm) splenomegaly, higher (>20) total symptoms score, presence of cytopenias, Charlson comorbidity index (>2) and body mass index (>21 and >30). In multivariate analysis, PMF diagnosis (HR 1.6 CI95% 1.07-2.5), age≥65 years (HR 2.1 CI95% 1.3-3.3) and previous infection (HR 3 CI95% 1.7-5.4%) confirmed their negative prognostic association. Interestingly, RUX dosage, spleen response and hematological toxicities during treatment were not associated with infectious risk.

Summary/Conclusions: Infections occurred in around one-third of RUX-treated pts; the rate of infections tended to decrease over time, and were fatal in 11% of the cases. Advanced age, a previous infectious event and diagnosis of PMF seem to be the main contributors to infectious risk.
ET) chose watchful waiting to manage >25% of their pts at diagnosis; 22% of untreated pts had moderate to high (quartiles 3-4) overall symptom burden. Physicians primarily recommended treatment for pts experiencing severe symptoms (72% MF, 68% PV, 72% ET) or symptomatic splenomegaly (71% MF, 61% PV, 39% ET). PLB was mainly used to treat pts with PV. Of those who received PLB (n=115), 71% were very or somewhat satisfied; 25% were very or somewhat dissatisfied and felt that PLB had a negative impact on their QOL. Similarly, 37% of physicians felt that PLB had a negative impact on pt QOL; PLB alone was insufficient for disease control in 38% of pts. Pts stopped PLB because physician deemed it no longer necessary (62%), pts felt worse after treatment (10%), and visit frequency was inconvenient (8%). Physician-reported reasons for stopping PLB were that visit frequency was inconvenient (38%), pts felt worse after treatment (35%), and lack of intravenous access (33%). HU use was assessed in pts with PV or ET. Of those who received HU (PV, n=95; ET, n=145), 78% and 74%, respectively, continued to receive HU; 19% and 22% were dissatisfied with HU therapy. Main reasons for stopping HU were lack of efficacy (29% PV, 13% ET) and toxicity (10% PV, 27% ET). Overall, 78% of physicians reported that up to 25% of their 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 therapy.

Summary/Conclusions: Many pts with MPNs are managed with watchful waiting at diagnosis. Although most of these pts have a low symptom burden, 22% have a moderate to high burden, highlighting the need for proactive and standardized symptom assessments at diagnosis and over the course of treatment. Individualized preferences and needs 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


Background: Ropeginterferon alfa-2b is a novel long-acting monopegylated interferon alpha (IFNa) with Orphan designation in Europe and the U.S. Reduced dosing frequencies and favorable tolerability accompanied by robust clinical responses in patients with polycythemia vera (PV) have been reported over the first few years of treatment. Successful long-term, potentially life-long maintenance with high rates of adherence, compliance and treatment outcome remain important goals to be elucidated.

Aims: PEGINVERA phase II (NCT: 2010-018768-18), is a prospective, open-label, multicenter study investigating efficacy and safety of ropeginterferon alfa-2b in long-term treatment of patients with confirmed diagnosis of PV, pre-treated or naïve to cytoreductive therapy.

Methods: All patients responding well to the initial bi-weekly administration or naïve to cytoreductive therapy. Patients continuing in the study for more than one year, had the option to switch to a “once every 4 weeks” schedule. The 2-week regimen (defined as the time period when all criteria for switching were fulfilled but the patient continued the 2-week regimen) was compared to the 4-week regimen (for a duration of 6 months after switch). The present analysis was focused on maintenance of efficacy.

Results: Data from the last available analysis include 29 patients remaining on study with a median treatment duration of 213 weeks. All 29 patients have completed at least 2 years of treatment (5 patients are in the 3rd year, 7 in the 4th year, 10 in the 5th year and 7 in the 6th year of treatment). Baseline characteristics of the study cohort and short-term treatment and individual adverse events and efficacy follow-up data were already presented earlier (Gisslinger et al. 2015). All of the 29 patients were switched to dosing every 4 weeks. Median treatment duration at time of switch was 104 weeks (Q1-Q3: 69-124 weeks). All 29 patients remained on the 4-week schedule with a median observation of 2 years. The 4-week regimen, reflecting an excellent safety and tolerability profile of ropeginterferon alfa-2b in this setting. The percentage of patients maintaining their best haematological response according to ELN before (i.e. after median 104 weeks of treatment) and 6 months after switching to the 4-week regimen was consistent at 51.7%. Further, need for phlebotomy during the 6 months after switching did not increase (consistent 7/29 patients). Changes in haematological parameters and spleen size were minimal and without clinical relevance. Similarly, the percentages of patients maintaining their best molecular response were 62.1% and 58.6%, respectively (non-significant). Importantly, the majority of patients on ropeginterferon alfa-2b long-term treatment, developed a sustained reduction of mutant JAK2 allele burden to below 10%, a feature that can only be achieved by IFNa based therapies.

Summary/Conclusions: In summary, all patients remaining on ropeginterferon alfa-2b after a median of 2 years of initial treatment were successfully switched to a more convenient monthly long-term maintenance schedule, thereafter no patients discontinued, and all patients could be maintained on this schedule for currently another 2 years (trial still ongoing). These data underscore the expected long-term efficacy with regard to haematological, clinical and molecular parameters and the excellent safety/tolerability of ropeginterferon alfa-2b. Long-term maintenance treatment of PV patients using ropeginterferon alfa-2b monthly is feasible, efficacious and well tolerable. Continuous patient-individual adaption of dosing regimen, including dose and dosing schedule, is recommended.

P708 NO IMPROVEMENT IN SURVIVAL OVER TIME FOR PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASM PATIENTS WHO TRANSFORM TO ACCELERATED OR BLAST PHASE

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Background: The outcome of patients with Philadelphia negative myeloproliferative neoplasms (MPN) who transform to acute leukemia is abysmal. There have been no advances in targeted therapy for this cohort of patients or individualized treatment based on genomic information. Furthermore, no large studies have investigated the impact of molecular profiling on clinical outcome in patients with accelerated or blast phase of MPN.

Aims: To describe the clinical outcomes of patients with MPN who transform to accelerated or blast phase and evaluate the impact of genomic alterations on outcomes.

Methods: Eligibility criteria included: Prior diagnosis of Philadelphia negative MPN according to WHO 2008 criteria; evidence of transformation to accelerated (10-19% blasts in peripheral blood or bone marrow) or blast phase (≥20% blasts) and seen at Princess Margaret Cancer Center between January 1998 and February 2017. The primary endpoint was overall survival (OS); defined as the time from transformation to death or last follow-up. Secondary endpoints included survival based on curative versus non-curative approach and treatment over time. In addition the impact of mutations will be correlated with clinical outcomes and survival.

In summary, all patients remaining on ropeginterferon alfa-2b after a median of 2 years of initial treatment were successfully switched to a more convenient monthly long-term maintenance schedule, thereafter no patients discontinued, and all patients could be maintained on this schedule for currently another 2 years (trial still ongoing). These data underscore the expected long-term efficacy with regard to haematological, clinical and molecular parameters and the excellent safety/tolerability of ropeginterferon alfa-2b. Long-term maintenance treatment of PV patients using ropeginterferon-alfa-2b monthly is feasible, efficacious and well tolerable. Continuous patient-individual adaption of dosing regimen, including dose and dosing schedule, is recommended.

Figure 1. Results: One hundred and eighty-seven patients who transformed to accelerated or blast phase with a prior diagnosis of MPN were identified at our insti-
Summary/Conclusions: Despite advances in systemic therapies and supportive care, there has been no significant improvement in survival for MPN patients who transform to accelerated or blast phase, confirming that current treatment approaches are ineffective. Results of molecular profiling may provide valuable insights and clues as to how to develop an individualized treatment approach for this cohort of patients.

Other Non-malignant hematopoietic disorders

P709

MASITINIB FOR TREATMENT OF SEVERELY SYMPTOMATIC INDOLENT SYSTEMIC MACROCYSTIS: ADDITIONAL EFFICACY ANALYSES FROM THE RANDOMIZED, PLACEBO-CONTROLLED, PHASE 3 STUDY

Background: Masitinib, a selective oral tyrosine kinase inhibitor targeting wild-type KIT, LYN and FYN, was the first drug to demonstrate efficacy in a phase 3 setting (study AB06006) for treatment of patients with severe indolent systemic mastocytosis (ISM) who are unresponsive to existing, optimal symptomatic treatments. In The Lancet (Feb 11;389(10069):612-620), Lortholary and colleagues reported a significant and clinically meaningful treatment benefit for masitinib (6mg/kg/day over 24-weeks) versus placebo, with primary analysis based on cumulative response (≥75% improvement from baseline, timeframe weeks 8-24, comprising 5 visits at 4-week intervals) in at least one of four severe baseline symptoms (pruritus, flushes, depression, or fatigue) using repeated measures methodology for rare diseases (i.e. a longitudinal analysis with respect to symptoms as opposed to patient response rate at a single point in time). Eligible patients were aged 18–75 years and had ISM according to inclusion criteria that were slightly broader than the WHO classification. Aims: To aid 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 mastocytis, 64 placebo), 90% of whom satisfied the WHO classification. Results: Masitinib showed a significant improvement over placebo according to its pre-specified primary endpoint (mITT population), with a cumulative response of 16.7% versus 7.4%, respectively, odds ratio (OR) of 3.6 [95%CI 1.2-10.8], P=0.008 (with re-randomization). This outcome was confirmed in the WHO patient subgroup: 17.8% versus 8.0%, respectively, OR=3.25 [0.97-10.88], P=0.0317. Computing the primary analysis (mITT) according to cumulative response per patient (GEE model) was also positive: 26.7% versus 12.8%, respectively, OR=2.48 [1.16-5.31], P=0.0212, as was analysis according to individual patient response (Pearson chi-square): 40.3% versus 24.2%, respectively, P=0.0062. Response (per patient) on all severe baseline symptoms for at least one visit was: 16.4% versus 7.6%, respectively, P=0.0062. Finally, analysis of sustained response in all severe baseline symptoms over multiple visits was highly discriminative between treatment arms: for patients with 3 severe baseline symptoms generated a 12.5% response rate (≥75% improvement in each symptom) for 3 out of 5 visits, versus no response for placebo; and for patients with ≥2 severe baseline symptoms masitinib generated a response rate of 21.1%, 15.8% and 10.5% over at least 1, 2, and 3 visits, respectively, versus no response for placebo. Summary/Conclusions: These post-hoc analyses confirm the clinical relevance, durability, and generalizability of the positive primary endpoint from study AB06006. Findings therefore support the conclusion that masitinib generates a significant therapeutic benefit in patients with severely symptomatic ISM who were unresponsive to optimal symptomatic treatments.

P710

THERAPY RESPONSE AND LONG-TERM OUTCOME OF 71 ADULT PATIENTS WITH HEMATOMALIGINANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: A SINGLE INSTITUTION EXPERIENCE

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Masitinib, a selective oral tyrosine kinase inhibitor targeting wild-type KIT, LYN and FYN, was the first drug to demonstrate efficacy in a phase 3 setting (study AB06006) for treatment of patients with severe indolent systemic mastocytosis (ISM) who are unresponsive to existing, optimal symptomatic treatments. In The Lancet (Feb 11;389(10069):612-620), Lortholary and colleagues reported a significant and clinically meaningful treatment benefit for masitinib (6mg/kg/day over 24-weeks) versus placebo, with primary analysis based on cumulative response (≥75% improvement from baseline, timeframe weeks 8-24, comprising 5 visits at 4-week intervals) in at least one of four severe baseline symptoms (pruritus, flushes, depression, or fatigue) using repeated measures methodology for rare diseases (i.e. a longitudinal analysis with respect to symptoms as opposed to patient response rate at a single point in time). Eligible patients were aged 18–75 years and had ISM according to inclusion criteria that were slightly broader than the WHO classification. Aims: To aid 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 mastocytis, 64 placebo), 90% of whom satisfied the WHO classification. Results: Masitinib showed a significant improvement over placebo according to its pre-specified primary endpoint (mITT population), with a cumulative response of 16.7% versus 7.4%, respectively, odds ratio (OR) of 3.6 [95%CI 1.2-10.8], P=0.008 (with re-randomization). This outcome was confirmed in the WHO patient subgroup: 17.8% versus 8.0%, respectively, OR=3.25 [0.97-10.88], P=0.0317. Computing the primary analysis (mITT) according to cumulative response per patient (GEE model) was also positive: 26.7% versus 12.8%, respectively, OR=2.48 [1.16-5.31], P=0.0212, as was analysis according to individual patient response (Pearson chi-square): 40.3% versus 24.2%, respectively, P=0.0062. Response (per patient) on all severe baseline symptoms for at least one visit was: 16.4% versus 7.6%, respectively, P=0.0062. Finally, analysis of sustained response in all severe baseline symptoms over multiple visits was highly discriminative between treatment arms: for patients with 3 severe baseline symptoms generated a 12.5% response rate (≥75% improvement in each symptom) for 3 out of 5 visits, versus no response for placebo; and for patients with ≥2 severe baseline symptoms masitinib generated a response rate of 21.1%, 15.8% and 10.5% over at least 1, 2, and 3 visits, respectively, versus no response for placebo. Summary/Conclusions: These post-hoc analyses confirm the clinical relevance, durability, and generalizability of the positive primary endpoint from study AB06006. Findings therefore support the conclusion that masitinib generates a significant therapeutic benefit in patients with severely symptomatic ISM who were unresponsive to optimal symptomatic treatments.
Background: Hemophagocytic lymphohistiocytosis (HLH) is a devastating disorder of uncontrolled immune activation characterized by clinicopathological evidence of extreme inflammation. Hematological malignancy-associated HLH (HM-HLH) has the worst outcome in comparison with any other form of HLH. HM-HLH can occur as the first manifestation of an occult malignancy, before start or during the treatment of known malignancy, or as the sign of a malignancy relapse or transformation to the more aggressive disease form.

Aims: The aim of the present study was to analyze the response to HLH therapy and overall survival of adult patients with HM-HLH.

Methods: From 2008 and onwards, data on adult patients referred to the Hematology Center Karolinska with suspected HLH were prospectively collected. Review concerned records of 142 adults with suspected HLH, hospitalized between January 2009 and December 2016. Of those, 71 patients with hematological malignancy were diagnosed with HLH and included to the present study. Hematological malignancy was defined as a neoplasm of lymphoid or myeloid origin. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Infection as a possible additional trigger of HLH was carefully studied in all our HM-HLH patients. EBV and CMV DNA were routinely examined in whole blood, using RT quantitative PCR; other viruses (e.g., adenovirus, HSV, VZV, HHV6, influenza) were studied based on indications. Blood and urine cultures were performed in order to reveal any bacterial or fungal infections. Tests for fungal antigens, tuberculosis, and parasites were also performed if indicated. HLH treatment categories have included proapoptotic chemotherapy (etoposide) and immunosuppressive drugs, targeting hyperactivated macrophages (etoposide, corticosteroids, IVIG) and T cells (corticosteroids, cyclosporine A).

Results: Seventy-one adults, aged 22–84 years, were diagnosed with aggressive HM-HLH during the 8-year period. Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of an unknown malignancy, during aggressive disease, or malignancy relapse. The remaining 24% patients developed HLH during chemotherapy. In 14 patients, HLH therapy started before confirmation of HLH diagnosis, based on suspicion of HLH (mean 6.7±8.4 days; range 0–41 days; median 2 days; range 1–31 days). Seventeen patients started HLH therapy at the day of HLH diagnosis. In 36 patients HLH therapy started after confirmation of HLH diagnosis (mean 15.9±4.13 days; median 5 days; range 1–242 days). Forty of 71 (56%) patients with active HLH died, of which 20 had signs of progressive malignancy. 16 patients had generalized infection (bacterial - 12 patients, viral - 3 patients, fungal - 4 patients; some patients had more than one type of infection), and 20 patients developed central nervous system bleeding. Thirty-one (44%) patients responded to HLH therapy and achieved remission of HLH. However, only 13 of 71 (18%) patients with HM-HLH were still alive after a median follow-up time of 50 months, despite the attempted treatment in 67 (94%) cases. The probability of overall survival (OS) from 0, 12, 24 and 60 months after HLH diagnosis was 39, 20, 15 and 15%, respectively. The patients who developed HM-HLH with concomitant infection during chemotherapy had significantly longer OS (p=0.03) compared to patients who had HLH solely attributed to malignancy (Figure 1).

Summary/Conclusions: HLH in the context of malignancy is still considered a challenging adult hematologic. HM-HLH is a highly lethal disorder in adults. The patients who develop HM-HLH with concomitant infection during chemotherapy show better survival than those who had HLH solely attributed to malignancy. Although poor outcome in some patients with HM-HLH is related to malignancy progression, in some patients the lack of effective M-HLH therapy may further impede adequate treatment of malignancy.

P711

WHOLE-EXOME SEQUENCING IN CHILDREN WITH IMMUNE CYTOPENIA: THE APPLICABILITY AND CLINICAL IMPACT

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Background: Next generation sequencing is rapidly becoming the main diagnostic tool for precise identification of gene defects in human diseases. Apart from the identification of novel causal genes and pathogenic variants, the main research goal for now is to assess the impact and strategy of whole exome sequencing (WES) use in routine clinical evaluation.

Aims: We aimed to evaluate the benefits and drawbacks of using WES as a diagnostic method in patients with chronic early-onset autoimmune hemolytic anemia (AIHA), idiopathic thrombocytopenic purpura (ITP) and immune neutropenia, or their combination (Evans syndrome). Most of these patients presented with additional symptoms of immune dysregulation, e.g., common variable immunodeficiency (CVID), lymphoproliferative, autoimmune disorders (diabetes mellitus 1, thyroëditis). We aimed to identify a novel variant leading to CTLA4 deficiency, another novel variant in CTLA4 was identified together with an additional pathogenic variant in TSC1, causing a mixed phenotype. The genetic diagnosis of CTLA4 deficiency allowed for the use of CTLA4 agonist (Abatacept) treatment in 1 patient that led to improvement of his symptoms and disease stabilization. However, after 6 months, the patient had developed agranulocytosis that led to hematopoietic stem cell transplantation. In 1 patient we were able to identify a gain-of-function variant in STAT3 that was recently described in immune dysregulation. In 3 patients we observed variants in genes typically described in connection with antibody deficiency (TACI, CD40L, and IKBKG). In 1 patient with chronic AIHA and ITP we found a novel heterozygous variant in TERT gene related to dyskeratosist congenita. In 1 patient with multiple congenital abnormalities and Evans syndrome we discovered a heterozygous variant in KMT2D gene causing Kabuki syndrome. The remainder of our patients harboured variants that posed a diagnostic challenge. In 4 of these we identified variants in genes involved in the pathogenesis of immune dysregulation, which are observed at lower frequencies also in healthy people (CASP10, PIK3CD). 12 patients (36%) had either only one hit in the genes reported causal in autosomal recessive diseases (e.g., ITK, LRBA) or we have not yet found any relevant aberration. In 4 patients we were able to identify novel variants in genes related to immune dysregulation. However, these variants require extensive validation studies, using patients’ primary cells or manipulating established in-vitro or animal models and gene editing techniques, in order to prove the causality.

Summary/Conclusions: WES is a highly useful method that helps to identify the genetic cause of the disease in approximately one third of patients and enables targeted therapy. While targeted sequencing can further reduce costs and make analysis more straightforward, gene panels are quickly becoming obsolete as new causal variants are discovered in the rapidly evolving field of primary immunodeficiencies. Because of the heterogeneity of genetic causes of immune cytopenias, we recommend to use WES over targeted gene panel sequencing.

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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 (Secondary Erythrocytosis). Secondary erythrocytosis related to lung, cardiac or renal disorder. Next generation sequencing (NGS) has been used to analyse the presence of mutations in 28 genes (enlarged hypoxia pathway and other candidate genes).

Methods: We created and developed a national network in France to analyze the genomic abnormalities in patients suspected of CE. The selection of patients was performed by clinicians and according to the hypoxia induced erythrocythemia Vossen criteria.

Results: To date, samples from 140 patients have been registered, among whom 46 have been analysed using NGS approach. Variants in 14 different genes were found in 14 patients. [13 males and 1 female; median age 50 y. (12-71)] with unknown significance have been detected, including 4 in PHD genes, 5 in HIF genes, 4 in LNK genes (SH2B3) and 1 in JAK2 gene. In patients with variants, a familial history of erythrocytosis was noted in 3. No independent thrombotic complication was reported in the patients. All patients were followed up for a median of 6 months (range 3 to 24 months).

Aims: To identify the specific PB cell compartments that carry this mutation.

Methods: The distribution of different maturation-associated of BM and PB CD34+ HPC from 64 newly-diagnosed (KIT-mutated) ISM patients and 14 healthy controls was analyzed by flow cytometry. In 18 patients distinct FACS-purified PB cell compartments were also investigated for the KIT mutation. Results: ISM patient’s showed higher percentages of both BM and PB KIT-mutated CD34+ HPC vs controls, particularly among ISM cases with MC-restricted KIT mutation (ISMmc); this was associated with progressive blockade of maturation of CD34+ HPC to neutrophil lineage from ISMMC to multilineage KIT-mutated cases (ISMkl). Regarding the frequency of KIT-mutated cases and cell populations in PB, variable patterns were observed, the percentage of KIT-mutated PB CD34+ HPC, eosinophils, neutrophils, monocytes and T-cells increasing from ISMMC to ISMkl to ISMMk patients.

Summary/Conclusions: Positive for the KIT D816V mutation in PB of ISM is associated with an expansion of circulating KIT-mutated cell populations and with multiple myeloid cell populations, KIT-mutated PB CD34+ HPC potentially contributing to disease dissemination already at very early stages.
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Background: Erythrocytosis, (i.e. increased levels of hemoglobin/hematocrit (Hb/Ht) >95% percentile 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 evidential 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.

Results: Patients were group according to the definitions of absolute Erythrocytosis. A total of 44 pediatric cases were identified (less than 18 years old). There were 7 families, where 5 adults were also found polyglobulic. However, in only 4 families a defect was identified 2 VHL, and 2 Hb variants). One Hb positive case was found sporadic. Most Hb variants were not symptomatic, while all other 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 VHL variant, who presented with arterial hypertension, a small size ganglioneuroma was found after a 5yrs follow-up. In 21 cases non causes could be identified. They were mostly male (n18); presented at adolescent age with advanced puberal status (n17); many were symptomatic (6). Only one 9 year old girl was diagnosed with polycytysma vera (JAK2V617F positive). Treatment varied according to physician decisions and presence of vascular symptoms, 6 children received ASA and 11 were phlebotomised. In two older patients severe vascular complications were observed (arterial thrombosis), in one with Htc<45%. Summary/Conclusions: This series shows the heterogeneity of Erythrocytosis as found in pediatrics. Extensive clinical and genetic analysis are required but still a large number of cases lack clear definitions. The usefulness of antigaeation and phlebotomy is not proved.

P717

AUTOIMMUNE NEUTROPEINIA OF CHILDDHOOD SECONDARY TO OTHER AUTOIMMUNE DISORDER: DATA FROM THE ITALIAN NEUTROPEINIA REGISTRY


Background: Neurological involvement is poorly described in autoimmune cytopenias (AIC), containing immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AIHA) and Evans syndrome (ES). This association suggests an underlying primary immunodeficiency (PID).

Aims: To describe neurological involvement observed in autoimmune cytopneas, evolution under treatment and PID profiles in these patients.

Methods: OBS'CREASEVANCE is a French nationwide prospective cohort including patients with a PID. Patients with a neurological involvement were analyzed excluding neurological symptoms related to a traumatism or isolated febrile seizures. Clinical, radiological and biological data were collected from primary centers. Centralized radiological review was performed. Genetic analyses were performed by Sanger gene sequencing or gene-panel based next generation sequencing.

Results: On October 2016, among the 1,167 patients of the cohort (371 AIHA, 615 ITP, 181 ES), a significant neurological involvement was observed in 8 patients from 7 centers. With a median (range) follow-up of 12 years (6-26.5), 7 children had ES (including autoimmune neutropenia in 5) and 1 child had isolated ITP. Median age at diagnosis was 11.5 years (1.6-15.8). At the last follow-up point, AIC were in partial or complete remission for all patients. Neurological symptoms appeared with a mean delay of 6 years (2.5-18) after AIC onset. The symptomatology was: seizures (n=4), cranial nerve palsy (n=2), Brown-Sequard syndrome (n=2) and/or sensory neuropathy (n=1). No infectious pathogens were identified. MRI showed multiple (n=6) or unique (n=2) inflammatory lesions with hypertense T2 signal in all patients, gadolinium-enhancing lesions in 7 and perilesional edema in 5. Five patients had a total of 8 biopsies, which confirmed the inflammatory process with macrophagic (n=3) or lymphoplasmocytary (n=5) infiltrates. In 4 cases, a lymphocytic meningitis was associated. Non-neurological organ involvement was present in all patients, mainly pulmonary nodules (n=6) and lymphoproliferation (n=4). All patients had an abnormal immunophenotype, with T-cell (n=7) or B-cell (n=3) deficiency and hypogammaglobulinemia was present in 7 of the 8 cases. Pathogen have been given standard treatment for autoimmune immunological (n=2), or immunosuppressive treatment (n=3, Ciclosporin, Mycophenolate Mofetil and Methotrexate), improving symptomatology and MRI for all. Five patients relapsed and 3 patients had an asymptomatic radiological progression. At the last follow up point, all patients had neurological sequel and 7 persisting radiological abnormalities. Four out of 8 patients analyzed had a PID: 22q11.2 microdeletion (n=1), heterozygous C7A mutation (n=2) or homozygous LRBA mutation (n=1).

Summary/Conclusions: Neurological involvement is a rare and severe late event in the course of childhood ES, or exceptionally AIHA, that may reveal various underlying PID. Complete imaging and pathology examination highlight a causative immune dysregulation and could guide specific therapeutic strategies.
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Background: The most frequent Autoimmune Neutropenia (AIN) in childhood is the primary type (p-AIN), whereas in adults AIN is mostly represented by secondary neutropenias, which can be associated to infection, drug administration, immunodeficiency, neoplasms, bone marrow transplantation or other autoimmune disorders.

Aims: To describe clinic and laboratory findings in children affected by AIN secondary to other autoimmune diseases (s-AIN).

Methods: This registry study analyzes 26 patients affected by s-AIN enrolled in the Italian neutropenia registry of A.I.E.O.P. (Associazione Italiana di Onco-Ematologia Pediatrica) over a 15-year time-span: this cohort, the largest ever described, was compared to 263 patients affected by p-AIN enrolled in the Registry in the same period.

Table 1.

Results: Specific characteristics of s-AIN patients are presented in Figure 1. The prevalence of former preterm babies among p-AIN (and not s-AIN) patients was significantly higher than in a cohort of 487 consecutively hospitalized children (p=0.0003). The median age of onset of AIN was 0.77 year and 10.07 year in p-AIN and s-AIN respectively (p=1.105e-12). The prevalence of selected IgA deficiency was 3% in p-AIN and 13.6% in s-AIN children: both prevalences were significantly higher than that (0.21%) of a group of 470 controls (p=0.0009 in p-AIN and p=7.239e-12 in s-AIN). Median value of neutrophils was lower in p-AIN (0.45 x 10^9/L) than in s-AIN 0.63 x 10^9/L (p=0.03); median value of lymphocytes was significantly reduced (p=6.29e-11) in s-AIN (1.58 x 10^9/L) vs p-AIN (4.36 x 10^9/L) group. Leucopenia (p=1.80e-07) and severe infections (p=0.0001) occurred more frequently in s-AIN; mononcytosis (p=0.039) and spontaneous remission (p=3.21e-11) in p-AIN. GCSF was used in 6.9% of the patients with eculizumab: abortion threat 35.3% vs 85.7%, fetal growth retardation syndrome 7.1% vs 21.4%, preeclampsia 5.9% vs 14.3%. Transfusion rate was higher without eculizumab (86.7% vs 41.2%). Pregnancies resulted in the birth in 100% patients exposed eculizumab and 42.9% on supportive treatment. Mean birth weight 2560 g (450-3550). Most of newborns (87.5%) are healthy, 83.3% of them received breastfeeding without complications both on eculizumab and without it.

Summary/Conclusions: p-AIN is in the vast majority of cases a benign and self-limiting disorder typically occurring under 2-3 years old whereas s-AIN is a more severe disease, usually appearing after the first 5 years of life, usually associated to lymphocytopenia and with a highly frequent tendency to become chronic.

P718 PAROSYMSAL NOCTURNAL HEMOGLOBINURIA TREATMENT DURING PREGNANCY

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a life-threatening disorder with a high risk of thrombosis. Targeted therapy radically changed the prognosis in PNH. Therefore issues of reproductive health in PNH patients are becoming very important. Recently the management of PNH during pregnancy has been challenging because of the high risk of maternal morbidity and frequent pregnancy loss. The combination of targeted therapy with eculizumab and anticoagulants made it possible not only to increase the survival rate, but also to improve the quality of life.

Aims: We compared the pregnancy outcomes in PNH patients on eculizumab treatment and retrospective data of pregnancies on symptomatic therapy only.

Methods: Since 1999 we have analyzed 32 pregnancies in PNH patients. 17 patients (group 1) from 2013 exposed to eculizumab during pregnancy with anticoagulants. Other 15 women (group 2) received only symptomatic therapy.

Results: Clinical manifestations of hemolysis significantly regressed during eculizumab treatment: normalization of LDH was registered in 76.5% patients. Without eculizumab LDH level increased in all pregnant patients. No maternal death and thrombotic events have been observed. 42.9% of patients required a dose adjustment due to breakthrough hemolysis (a dose increase and/or more frequent use of eculizumab). Pregnancy complications were less frequent with eculizumab: abortion threat 35.3% vs 85.7%, fetal growth retardation syndrome 7.1% vs 21.4%, preeclampsia 5.9% vs 14.3%. Transfusion rate was higher without eculizumab (86.7% vs 41.2%). Pregnancies resulted in the birth in 100% patients exposed eculizumab and 42.9% on supportive treatment. Mean birth weight 2560 g (450-3550). Most of newborns (87.5%) are healthy, 83.3% of them received breastfeeding without complications both on eculizumab and without it.

Summary/Conclusions: We can conclude that pregnancy outcomes in PNH patients with eculizumab are much better than with symptomatic therapy only. Our data demonstrate the possibility of safe therapy with eculizumab in pregnant women. Pregnancy does not worsen the prognosis of PNH in the case of targeted and adequate supportive therapy. There is no difference in health between infants born by mothers with PNH and the newborns from general population.
Platelet disorders: Clinical

P719
LONG-TERM RESPONSE TO ORAL ELTROMBOPAG IN TREATMENT-NAÏVE ADULTS WITH GAUCHER DISEASE TYPE 1: FINAL EFFICACY AND SAFETY RESULTS FROM A PHASE 2 CLINICAL TRIAL AFTER 8 YEARS OF TREATMENT

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Background: In Gaucher disease type 1 (GD1), deficient lysosomal acid β-glucosidase activity leads to accumulation of glucosylceramide, primarily in macrophages (Gaucher cells), which deposit in the spleen, liver, and bone marrow, leading to thrombocytopenia, anemia, hepatosplenomegaly, and skeletal disease. Hematologists often identify and manage the disease. Intravenous enzyme replacement therapy (ERT) with recombiant acid β-glucosidase has been the mainstay of therapy for GD1. Eltrombopag is an oral oral platelet-stimulating agent approved as first-line treatment for adults with GD1 with poor, intermediate, or extensive CYP2D6-metabolizer phenotypes (>90% of patients). Phase 3 trials demonstrated safety and efficacy of eltrombopag in naïve patients (Mistry et al. JAMA. 2015) and safety and stability in patients switching from long-term ERT (Cox et al. Blood. 2017). We report the final 8-year results of an open-label Phase 2 trial (NCT00385180, Sanofi Genzyme) in previously untreated adults with GD1. These data build on 1-, 2-, and 4-year data showing sustained improvements in hematologic parameters, organ volumes, disease-related biomarkers, and measures of bone health (Lukina et al. Blood Cells Mol Dis. 2014).


Methods: Adult GD1 patients who had splenomegaly with thrombocytopenia and/or anemia received 50 or 100mg eltrombopag twice daily, dosed by plasma trough levels. Efficacy outcomes included changes in hemoglobin, platelets, spleen and liver volumes, disease-related biomarker levels, skeletal manifestations, and achievement of therapeutic goals for anemia, thrombocytopenia, splenomegaly, and hepatomegaly (Pastores et al. Semin Hematol. 2004; Lukina et al. Blood. 2010).

Results: Of 26 enrolled patients, 19 completed the trial and 7 withdrew: 2 on the first day of treatment due to asymptomatic nonsustained ventricular tachycardia detected during routine monitoring (plasma levels of eliglustat were undetectable); 1 after 1 year due to progression of a bone lesion (retrospectively identified at baseline); 1 chose to withdraw after 2 years; and 3 due to pregnancy. After 8 years of eliglustat, mean (±SD) hemoglobin level and platelet count increased by 2.1±1.7 g/dL (from 11.3±1.6 to 13.4±1.3 g/dL) and 110% (from 76±52 to 158±96 x10^9/L, respectively). Mean spleen and liver volumes (multiples of normal, MN) decreased by 68% (from 17.3±10.4 to 5.1±3.5 MN) and 31% (from 1.6±0.5 to 1.1±0.3 MN), respectively. All patients met ≥3 of 4 long-term therapeutic goals (spleen, 100% of patients; liver, 100%; hemoglobin, 93%; platelets, 53%) by 7-8 years. Median chitotriosidase levels decreased by 43% with eliglustat (82%); α-L-fucosidase by 44% (88%); plasma GL-1 normalized. Total mean lumbar spine bone mineral density increased by 0.12 g/cm²; mean Z-score increased by 0.88 (from -1.27±1.02 to -0.39±1.13) and mean T-score by 0.95 (from -1.64±1.07 to -0.69±1.31). Eltrombopag was well-tolerated. All quality of life measures (SF-36, fatigue severity score) showed improvement over time. Most adverse events in this long-term trial were mild or moderate in severity (98%, 342/348) and considered unrelated (94%, 328/348) to treatment.

Summary/Conclusions: After 8 years of treatment with eltrombopag, clinically meaningful improvements in hematologic, visceral, biomarker, and bone parameters continued or were maintained among patients in this Phase 2 trial. No new safety concerns emerged.

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REAL WORLD EVIDENCE ON DRUG UTILIZATION PATTERNS OF ELTROMBOPAG IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIA: AN ANALYSIS OF ELTROMBOPAG [ELTROMBOPAG] IN SELECTED COUNTRIES IN THE EUROPEAN UNION (EU) STUDY

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Background: Immune thrombocytopenia (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia, with platelet counts <100x10^9/L. Ertrombopag is an oral small-molecule nonpeptide thrombopoietin-receptor agonist that has shown to increase platelet production in clinical trials. Ertrombopag is approved for 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 pediatrics aged 1-5 years, and 50mg OD (East Asians, 25mg OD) in adults and pediatrics aged 6-17 years at initiation, followed by dose adjustment to a maximum of 75mg OD based on platelet counts. REVIEU study was conducted in accordance with risk management plan in five European Union (EU) countries to document eltrombopag utilization patterns in real-world practice. Here, we report the eltrombopag prescribing 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), Greece (n=36), France (n=29), and Germany (n=27). Ertrombopag 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, 74 [25.6%]; corticosteroids+immunoglobulins+intravenous immunoglobulins, 114 [39.9%]). 126 (43.7%) patients received three prior therapies (corticosteroids, immunoglobulins, and splenectomy). In total, the majority of patients received at least one prescription of corticosteroids (252, 88.1%) followed by immunoglobulins (180, 62.9%), and splenectomy (64, 22.4%) prior to eltrombopag initiation. Patients received an average daily dose of eltrombopag 45.6mg (chronic ITP, 44.6mg; persistent ITP, 43.1mg; acute ITP, 53.0mg) during the study. Overall, dose changes were reported in 749 adult ITP prescriptions (down-titration, 53.7%; up-titration, 43.7%; no change in dose, 2.7%). 49.1% of dose changes were reported during the first 6 months of treatment (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 chronic ITP and 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. Ertrombopag was also prescribed in 24.4% of adult patients with acute and persistent ITP. The starting dose followed the summary of product characteristics (SmPC) recommendations in the majority of cases and dose modifications were generally according to platelet counts. Data from REVIEU study have shown that eltrombopag use in the real world setting is largely consistent with the EU label and is considered part of ITP medical therapies.
Background: A previous study has suggested a mechanism of Fcγ receptors (FcγR)-independent platelet clearance in immune thrombocytopenia (ITP) (1). Antibodies that related 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 treatments (corticosteroids, IVIG and/or splenectomy) as well as in 8 patients with non-refractory ITP (control group).

Results: The characteristics of the patients according to the response to conventional treatments (A, no responders; B, responders) are summarized in Table 1. Non responders exhibited lower platelet counts (p=0.006), higher expression of GPIIb (p=0.049) and loss of platelet sialic acids (p=0.005). Additionally, those who did not respond not only to traditional therapies (corticosteroids, IVIG and splenectomy) but also to thrombopoiesis. TPO-RAs–romiplostim (R) and eltrombopag (E) - offer a significant higher platelet desialylation in ITP patients who are non-responders to conventional therapies, par-

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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group A</th>
<th>Group B</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>60.2</td>
<td>52.1</td>
</tr>
<tr>
<td>Platelet count (×10^4/μL)</td>
<td>80.1</td>
<td>300.2</td>
</tr>
<tr>
<td>GPIIb expression</td>
<td>0.049</td>
<td>0.006</td>
</tr>
<tr>
<td>Sialic acid loss</td>
<td>0.005</td>
<td>0.006</td>
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Reference
Results: A total of 548 pts received either R or E between Dec 2009 and Dec 2015. Of these, 106 (19.4%) underwent TPO-RA switch. Table 1 summarizes outcome after switch. Overall 69/106 (65%) of pts achieved, regained or maintained response upon switching. Either one TPO-RA switch sequence was equally effective (p=0.882). Outcome was not associated with gender, age at 1st TPO-RA treatment, splenectomy status. However, number of lines of previous therapies interrupted or lower response (p=0.020); response on day 0/6 of line of therapy yielded a 30% increase in the odds of being a non-responder; a trend toward lower probability of response was observed in pts with longer lasting disease before 1st TPO-RA administration (p=0.066). Adverse events (AE; 16/106 pts) were generally mild and reversible upon discontinuation of either one TPO-RA. 1st TPO-RA was followed with thrombopoietin/erythropoietin (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 strategy. Exposure to the 2nd TPO-RA was more effective in pts who had lost response to 1st TPO-RA (80% responders) compared to those who were non responders to 1st TPO-RA (49% responders, p=<0.001). It could be speculated that lack of response to either one of the two available TPO-RA identifies a subgroup of pts less likely to benefit from switching to the second available TPO-RA. Pts switched for non-efficacy reasons are more likely to maintain a response upon switch (p=0.030). The so far unexplained and unprecedented phenomenon of wide plt fluctuation appears to be linked to the removal of the spleen, the physiological plt reservoir organ.
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 among severe (non-ICH) patients (P<0.009, RR=5.543, 95% CI, 1.317-15.668).

Summary/Conclusions: Platelet count <10×109/L, female patients, complication of pulmonary disease, gum or oral mucosal bleeding and epistaxis are significant predictive factors for severe bleeding in the elderly. Severe bleeding in elderly ITP was associated with more failure of response to treatment, increased long-term risk of no remission and mortality related to fatal bleeding.

P725
ATORVASTATIN IMPROVE THE PROGNOSIS OF ADULT PATIENTS WITH CORTICOSTEROID-RESISTANT IMMUNE THROMBOCYTOPENIA VIA ENHANCING BONE MARROW ENDOTHELIAL CELL FUNCTION

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Background: Immune thrombocytopenia (ITP) is generally considered to be an autoimmune disorder characterized by increased peripheral platelet destruction and reduced platelet production. Corticosteroids represent the standard first-line therapy achieving responses in around 80% of patients. However, for those corticosteroid-resistant ITP patients, who exhibit either no response (NR) to corticosteroids or corticosteroid-dependent, the pathogenesis remains poorly understood and the management is challenging. Emerging evidence from mouse studies has suggested that the cross-talk between megakaryocytes (MKs) and endothelial progenitor cells (EPCs) in the bone marrow (BM) microenvironment regulates MKs maturation and thrombopoiesis. We recently reported that the impaired BM EPCs, which could be quantitatively and functionally improved by atorvastatin in vitro, induced the occurrence of poor graft function following allo-transplant (Blood, 2016, 128:2988-2999). However, little is known about the functional role of BM EPCs and how to improve impaired BM EPCs in patients with corticosteroid-resistant ITP.

Aims: To determine whether quantitative and/or functional abnormalities of BM EPCs are involved in the occurrence of corticosteroid-resistant ITP. Moreover, to investigate the effects of atorvastatin and N-Acetyl-L-cysteine (NAC, a ROS scavenger) on the number and function of cultured 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 previous reported. Atorvastatin and NAC were administrated to the 5-day cultivated BM EPCs in corticosteroid-resistant ITP patients until tested on day 7. The number and function of BM EPCs were evaluated pre- and post-treatment, counting Dil-Ac-LDL and FITC-UEA-1 double staining, migration, cell proliferation, tube formation, levels of reactive Oxygen Species (ROS) and apoptosis. Proteins expressions for p38, ERK, JNK, Akt were measured by flow cytometry and western blot. Subsequently, a single-center pilot study was performed to evaluate the efficacy and safety of atorvastatin and/or NAC in corticosteroid-resistant ITP patients. The primary endpoints were complete response (CR), response (R), and overall response (OR). Secondary end points were time to response (TTR) and adverse events.

Results: Human bone marrow EPCs were demonstrated as the spindle shape and the similar expression of CD34, VEGFR2 and CD133 at day 7 of cultivation among patients. We enrolled three cohorts of subjects: increased and dysfunctional BM EPCs, which were characterized by impaired proliferation, migration, angiogenesis, and higher levels of ROS and apoptosis, were revealed in corticosteroid-resistant ITP patients compared to those in newly diagnosed ITP. Activation of p-p38 was detected in BM EPCs from corticosteroid-resistant ITP patients. Furthermore, the number and function of BM EPCs derived from corticosteroid-resistant ITP patients were enhanced by atorvastatin or NAC treatment in vitro through down-regulation of the p38 mitogen-activated protein kinase (MAPK) pathway. In the single-center pilot study, a total of 12 corticosteroid-resistant ITP patients were recruited to receive either the combination of atorvastatin and NAC or alone. ADR of CR, R and OR resulted from (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 in vitro and in vivo via down-regulation of the p38 MAPK pathway. Although the sample size of clinical study is small, with a relatively short follow-up period by now, our data suggest that atorvastatin and NAC are effective and safe in the management of corticosteroid-resistant ITP patients. Therefore, further prospective multicenter randomized clinical trials with larger sample size are needed in the future.
**Results:** The platelet desialylation levels increased significantly in the 127 septic patients with thrombocytopenia compared to the 134 patients without thrombocytopenia. A platelet response was achieved in 45 of the 54 patients in the oseltamivir group (83.3%) compared with 34 of the 52 patients in the control group (65.4%; \(P=0.045\)). The median platelet recovery time was 5 days (interquartile range 4–6) in the oseltamivir group compared with 7 days (interquartile range 5–10) in the control group (\(P=0.003\)). The amount of platelets transfused decreased significantly in the oseltamivir group compared to the control group (\(P=0.044\)). The multivariate analysis by Cox proportional hazards models showed that the Sequential Organ Failure Assessment (SOFA) score and platelet recovery time were independent indicators of oseltamivir therapy.

**Summary/Conclusions:** Thrombocytopenia was associated with increased platelet desialylation in septic patients. The addition of oseltamivir could significantly increase the platelet response rate, shorten platelet recovery time and reduce platelet transfusion. Chinese Clinical Trial Registry, ChiCTR-IPR-16005542.

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**PT27**

**SAFETY AND EFFICACY OF LONG-TERM OPEN-LABEL DOSING OF SUBCUTANEOUS (SC) ROMIPLOSTIM IN CHILDREN WITH IMMUNE THROMBOCYTOPENIA (ITP)**

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**Background:** Children with ITP for ≥6 months who completed a romiplostim phase 1/2 or phase 3 parent study could enroll in this open label long term extension study

**Aims:** To evaluate the safety and efficacy of long-term romiplostim in children with ITP.

**Methods:** Patients enrolled at 28 sites in the US, Canada, Spain, and Australia. All patients received SC romiplostim once weekly. The initial dose was the final dose from the parent study or 1 µg/kg for patients previously receiving placebo; dose was then adjusted from 1–10 µg/kg to target platelet counts of 50–200×10\(^9\)/L. Incidence of adverse events (AEs) was the primary endpoint.

**Results:** As of 24 Feb 2016, 66 patients entered this study; 65 received romiplostim for up to 6.2 years. At baseline, median (min–max) age was 11 (3–18) years; 56% were female; 61% were white, 14% African American, 14% Hispanic/Latino, 9% Asian, and 3% other; 9.1% had prior splenectomy. Median (min–max) baseline platelet count was 27.5 (2–458)×10\(^9\)/L. Median (min–max) treatment duration was 100 (5–321) weeks. Median (min–max) average weekly romiplostim dose was 4.8 (0.1–10.0) µg/kg, which included escalation to a stable dose. After ~week 200 (n ≤8 patients), the median dose was observed to fluctuate. All 65 patients received their doses per protocol >90% of the time; 18 patients missed ≥1 dose due to noncompliance for a total of 41 times. Reasons for discontinuing treatment (n=22, 33%) included consent with-
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. A mean change from BL to each assessment through Week 48 are reported here. Clinical relevance was based on minimum important difference (MID) of values from BL to each assessment. A change of 5-10 points is considered a "small" change on the EORTC-QLQ-C30. The lower bound of 5 points was used for MID acceptance on both measures.

Results: In this interim analysis, HRQoL improvements from BL were observed early and were sustained throughout the 48 weeks 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).

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 LEUKEMIA (CML) PATIENTS: IMPLICATION FOR MEDICAL DECISION MAKING

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1The University of Texas MD Anderson Cancer Center, Houston, 2AbbVie Inc, North Chicago, United States

Background: The prognosis for patients with CML after B-Cell Receptor inhibitor (BCR) failure is very poor. Patients with R/R CML who discontinue and/or progress on BCR 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) CML patients.

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Summary/Conclusions: This interim analysis provides preliminary evidence that demonstrates CML 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 CML following relapse or progression on BCRi inhibitors.

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 expected offering a more affordable treatment option for CLL patients across Europe.

Aims: To assess the budget impact of the introduction of CT-P10 into the treatment of CLL in the 28 EU member states. Moreover, we provide an estimation 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. 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), and Italy (€1.25 million). The budget impact per million patients treated is €12,000. The potential cost savings are in a direct correlation with the price and market uptake of CT-P10. Applying a 40% and 50% discount in drug prices compared to the originator rituximab, cost savings are projected to €23.73 and €29.67 million, from which further...
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 how remote monitoring contributes to 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 suitable options for remote monitoring (55% preferred a nurse-led telephone consultation (TC) replacing current face-to-face (FTF) consultations with a doctor. Median age was 61 years (range 46–76), and 9 were male. 18 patients were in 1st remission; 16 had most recently received high dose therapy and autograft, 3 had post autograft consolidation. The centre was not the local hospital for 18 patients interviewed. The majority were accepting of TC as an alternative to FTF clinics due to the burden of travel, associated cost and clinic waiting times. These affected patients’ physical and psychological well-being, with TC perceived as less 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. Patient’s suggested this service change would be beneficial for healthcare resourcing rather than themselves 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 conclusion that results of blood tests for MM were usually 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 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.

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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 and need to be interpreted with caution. We will seek to estimate radiation doses to the red bone marrow, based on limited data available on CT examinations (body part and examination type).

**References**

**P735**

**HEALTHCARE RESOURCE UTILIZATION WITH IXAZOMIB OR PLACEBO PLUS LENALDIDOMIDE-DEXAMETHASONE IN THE RANDOMIZED, DOUBLE-BLIND, PHASE 3 TOURMALINE-MM1 STUDY IN RELAPSED/ REFRACTORY MULTIPLE MYELOMA (RRMM)**

**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 (Gol 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 end-point was PFS. HRU was assessed on day 1 of each cycle prior to treatment and every 4/12 weeks during PFS/overall survival follow-up. After a median follow-up of ~23 months, pts had received a median of 17 (range 1-34) and 15 (1-34) cycles of ixazomib-Rd and placebo-Rd, respectively; HRU data are reported from this analysis time point.

**Table 1.**

**Table: Odds ratios and frequencies of CT scans**

**Methods:** We used nationwide, register-based case-control study design to investigate the role of CT imaging in the etiology of childhood leukemia. We identified all childhood (0-15 years) leukemia cases from 1990 to 2011 (N=10953) in Finland and randomly selected thrice as many controls (N=3279) from the Population Registry, individually matched by gender and year of birth. The cases were 81% (N=885) acute lymphoblastic leukemias and 13% (N=142) acute myeloid leukemias. We collected data on all pediatric CT scans from 1975 to 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
Results: Overall, 152 (42%) pts on the ixazomib-Rd arm had 316 hospitalization events, compared to 156 (43%) pts (335 events) in 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 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 4128 (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 ixazomib-Rd triple was consistent with the limited additional toxicity burden and the reported lack of an adverse impact on QoL with ixazomib-Rd. In contrast to findings reported for injected agents (Armoiry et al, J Clin Pharm Ther 2011; Gaultney et al, J Clin Pharm Ther 2013; Baz et al, Support Care Cancer 2015), this all-oral triplet regimen did not increase time lost from work, caregiver burden, or the number of inpatient/outpatient visits.

P737
EFFECT OF IMPROVEMENTS IN SURVIVAL, POPULATION AGING AND IMWG’14 CRITERIA ON INCIDENCE AND PREVALENCE OF MULTIPLE MYELOMA
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Background: There are some variables that can modify Multiple Myeloma incidence of New Diagnosed (NDMM) and prevalence over the time: Past decade shows a new demographic data in our society: the increment of expectancy of life and an excellent performance status. In the last years we have assisted to an amazing improvement in the management and expectancy of life of Multiple Myeloma (MM) patients. Recent changes in criteria recommendation by IMWG’14 to begin treatment in NDMM patients can increment its incidence. New expensive but very effective and well tolerated antilymoma (antiMM) agents are in the center of attention of Hematologic and Public Healthcare Systems. There are data of improvement of survival that can increment of prevalence.

Aims: We have analysed our data base and calculate incidence by sex, age and three 5-years periods of time at diagnosis and obtain tendencies to get ready for next decade of ageing people with best antilymoma agents. We have analysed prevalence of MM patients on last 7 years with cutoff date on 1st of November (2010 to 2016).

Methods: We retrospectively analysed the incidence of patients with new diagnostic of Multiple Myeloma (NDMM) from 1998 to 2012. (Fig.1). Then we divide the cohort in several groups: sex and age at diagnosis (3 groups: <65, 66-75 and 75+); and in four 5-year (quinquennium) period of time (1998-2002, 2003-07, 2008-12, 2013-NOV2016). (Fig. 2). We have calculated the incidence per 100000 inhab/year using census data of our Local Registry of Tumours of our Public Health Area. Characteristics of patients: n= 346. M/F: 206/140. Median age at diagnosis: 74 years (Range: 39-100).

Results: A) INCIDENCE RATES (see Table). In the past IMW (Roma-14#PO197) we reported incidence rates form 1998 to 2012. We observed a constant increase of Annual Average of incidence from 4.57 cases/100000 inhabitants/ year from the 1st period to 6.15 in the last. Adjusted by Age Incidence increase from 14 to 18.5 cases in the O65 group. From 2013 to Nov-2016 global and adjusted by age incidence remains similar to last years data with 80 new cases in the 4 year-period (5.9 cases for global population and 17.2 cases for over65 population). After IMWG’14 criteria to begin treatment in NDMM the incidence was similar to the last 7 years (2008-12 period) incidence with 37 NDMM cases (25 O65y group).

B) PREVALENCE RATES (PreVR).
• 2012. 77 pts alive. PreVR: 22.2 /100000 inhabit;
• 2014. 84 pts alive. PreVR: 24.4/100000 inhabit;
• 2016. 103 pts alive. PreVR: 30.3 /100000 inhabit.

Table 1.

Summary/Conclusions: Although we don’t observe substantial changes on incidence rates of NDMM, we have noted an important rise on prevalence rates of more than 40% from 2010 to 2016 (21.2 to 30.3 pts alive /100000 inhabit.) Several new antiMM drugs are available in the therapeutic arsenal and probably increases the prevalence rates.
Hematopoietic stem cell transplantation (HSCT) is a powerful tool in the treatment of hematologic malignancies, particularly in children with acute lymphoblastic leukemia (ALL) in first remission (iEMR). The decision to proceed with HSCT is based on several factors, including the patient's age, disease status, and the presence of risk factors. HSCT can be performed using autologous, allogeneic, or haploidentical donors, with each option having its own advantages and considerations.

In a recent study, 281 patients with ALL iEMR were included in the analysis. The patients were transplanted with allogeneic hematopoietic stem cell transplantation (HSCT) from 19 centers, members of the Italian Pediatric Onco-Hematology Association (AIEOP). The study aimed to evaluate the impact of different conditioning regimens on overall survival (OS) and the incidence of hepatic veno-occlusive disease (VOD) or sinusoidal obstruction syndrome (SOS), which are significant complications after HSCT.

Methods:
- Data were collected from 19 centers in Italy, members of the Italian Pediatric Onco-Hematology Association (AIEOP).
- Patients with ALL iEMR were included in the analysis.
- The study included 198 patients who underwent autologous HSCT (Auto HSCT) and 83 who underwent allogeneic HSCT (allo HSCT).
- Conditioning regimens included total body irradiation (TBI), fludarabine-based, busulfan-based, and cyclophosphamide-based regimens.
- The primary outcome was overall survival (OS) and the incidence of VOD or SOS.

Results:
- Overall survival (OS) for the entire cohort was 72%, with 5-year OS rates of 74% for the allo HSCT group and 69% for the auto HSCT group.
- The incidence of VOD/SOS was 11% in the allo HSCT group and 3% in the auto HSCT group.
- Patients who received a busulfan-based transplant had a lower risk of VOD/SOS compared to those who received a fludarabine-based transplant (HR 0.39, 95% CI 0.2-0.8).
- Age, site of relapse, length of first remission, HSCT type (Auto vs MFD), and receiving a busulfan-based transplant (TBI) were significant predictors of VOD/SOS.

Conclusion:
- The study highlights the importance of conditioning regimens in the prevention of VOD/SOS after HSCT.
- Busulfan-based conditioning regimens are associated with a lower risk of VOD/SOS compared to fludarabine-based regimens.
- Age, site of relapse, and length of first remission are significant predictors of VOD/SOS.

References:

Keywords:
- Hematopoietic stem cell transplantation
- Veno-occlusive disease
- Sinusoidal obstruction syndrome
- Hematologic malignancies
- Acute lymphoblastic leukemia
- Conditioning regimens
PAROXYSMAL NOCTURNAL HEMOGLOBINURIA-12

ALLO-HCT FOR PAROXYSMAL NOCTURNAL HEMOGLOBINURIA-12

**Background:**
Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal abnormality of hematopoietic stem cell leading to lack of phosphatidylinositol-4,5-bisphosphate 3-kinase C2 (PI3K-C2) activity. The disease is characterized by a complement-mediated intravascular hemolysis in the presence of a specific complement regulatory factor, leading to hemolytic anemia, microangiopathic hemolytic anemia, transfusion dependency, hemolytic crises, and thrombosis. The disease is caused by somatic mutation of the CYTL1 gene, which encodes a PIG-A enzyme (phosphatidylinositol glycan anchor biosynthesis class A) responsible for N-glycosylation of glycoproteins, necessary for proper cellular function. The main treatment for PNH is treprostinil, which reduces endothelial cell activation and improves hemodynamic features. However, this treatment is not curative, and there is a need for a curative option, such as allogeneic hematopoietic stem cell transplantation (allo-HCT).

**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 5% with MUD group, 9324 (38.0%) were pediatric (aged <16 years; 5105 [53.7%] with MOD) and 169264 (64.0%) were adults (aged >16 years; 88169 [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, 49.4%–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, 40.9%–56.0%) overall and 43.0% (95% CI, 32.5%–53.0%) for pts with MOD. Adverse events (AEs) occurred in 75.4% of the total group (80.6% with MOD); 70.5% of pediatric pts (76.6% with MOD); 78.1% of adults (83.0% with MOD). Treatment-related AEs (TRAEs) occurred in 20.8% overall (23.7% in those with MOD); 21.1% of pediatric pts (23.5% with MOD); 20.7% of adults (23.9% with MOD). The most common TRAEs (>3%) were epistaxis, pulmonary hemorrhage, gastrointestinal hemorrhage, and hematuria (each in <5% of pts). TRAEs leading to study discontinuation (n=25) or death (n=10), the most common was pulmonary hemorrhage.

**Results:**
All pts engrafted, median counts of granulocytes 0.5 G/l, platelets 50 G/l and Hb 10 g/dl were achieved on days 17.5(10-33), 16(9-39) and 19.5(11-34). Acute GVHD grade I and II was present in 16, 7 and 3 pt, limited and extensive chronic GVHD respectively in 11 and 3 pts. LDH decreased by 73%(5%-91%) in first 30 days indicating disappearance of hemolysis. 100% donor chimerism was achieved in all pts. In 1 patient donor chimerism decreased to 81% what was treated with donor lymphocytes infusion (DLI). 3 patients died, 1 previously hemolyzed pt died on day +102 due to nephrotoxicity complicating adenosine/CMV hemorrhagic cystitis, two other SAA patients with PNH clone<10% died on days +56 due to severe pulmonary infection and +114 due to aGVHD-III and multi organ failure. Complications in survivors were FVO (10 pts), CMV reactivation (13), VOD (1), neurotoxicity (1), acute graft-versus-host disease (1), hemolytic disease of the newborn (1) and mucositis (1) were present. Acute GVHD grade III was present in 17, 7, and 3 pt, limited and extensive chronic GVHD respectively in 11 and 3 pts. LDH were significantly lower compared to that of the MSD group (29 yrs, 31 yrs, and 39 yrs; P<0.01, and 44.6% and 33.3% vs. 89%; P<0.01, respectively). When we compared the incidence of transplant-related mortality (TRM) 10.7% vs 7.4% at 6 yrs; P=0.53) and overall survival rate (OS; 89.3% vs 92.5% at 6 yrs; P=0.52) between the WM-URD and the MSD groups, there were no significant differences. However, trends of higher TRM incidence (18.2% vs 7.4% at 6 yrs; P=0.05) and lower OS rate (81.8% vs 92.5% at 6 yrs; P=0.05) were observed between the PM-URD and the MSD groups. There was no primary graft failure in 38.6% of pts. Incidence of graft-versus-host disease of both grades in 0% vs 18.3%; P<0.01 and PM-URD (0% vs 18.3%; P=0.02) groups were significantly lower compared that of the MSD group. When we adjusted other clinical and transplant-related factors, which include age and IST failure, using multivariate
analysis, the OS rate of the WM-URD group was not significantly different (HR 1.45, 95% CI; 0.52-4.09; P=0.48), whereas that of the PM-URD group was significantly lower (HR 2.85, 95% CI; 1.01-8.02; P=0.04), compared to that of the MSD group.

Summary/Conclusions: Our study showed that there was no significant difference in OS rate between the WM-URD and the MSD groups. As high incidence of GVHD remains a problem in the former group, strategies to reduce it are needed in future protocols.

P743
HAPLOIDENTICAL ALLOGENEIC STEM CELL TRANSPLANTATION 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) 10mg/kg on days SCT -12,-11,-10, Flu 35mg/m2 on days SCT -7,-6,-5,-4,-3,-2 and IV Busulfan (Bu) 130mg/m2 on days SCT -7,-6,-5,-4 was given followed by T-cell replete peripheral blood progenitor cells (PBPC). GVHD prophylaxis consisted of cyclophosphamide (Cy) 50mg/kg on days SCT -3 and -4 (Post-Cy), and on day SCT +5 tacrolimus or sirolimus was started together with a short course of mycophenolate moefetil.

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 ADDITIONAL 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 (186mg/m²) (Flu-Bu), has reduced toxicity, however with the limitation of increased relapses. We have tried to improve outcome of Flu-Bu regimen by augmentation with Thiotepa (10mg/kg). Here we compared outcomes of 45 such patients (getting augmented regimen, Flu-Bu with the addition of Thiotepa, (group 2), to 44 patients who received Fludarabine, Busulfan myeloablative reduced toxicity regimen (group 1), during the same period

Aims: The primary objective of the report was to compare the toxicity and incidence of relapse between the two regimens. Secondary objective was to compare overall survival (OS), and disease-free survival (DFS), the non-relapse mortality (NRM), engraftment kinetics, incidence of acute and chronic graft-versus-host disease (GVHD), and comparison between high and low-risk patients amongst the two groups.

Methods: 89 patients with AML were retrospectively analyzed. 44 patients were conditioned with Flu-Bu (group 1) and 45 patients augmented with Thiotepa (Flu-Bu-TT, group 2). The transplant conditioning regimen, (augmented myeloablative) consisted of 30mg/m² intravenous Fludarabine for 5 days (total dose 150mg/m²), for matched related donors or for 6 days (180mg/m²), for unrelated or mismatched donors, intravenous Busulfan (3.2mg/kg/day for 4 days, total dose 12.8mg/kg), and intravenous Thiotepa 5mg/kg for 2 days (10mg/kg). The conventional myeloablative regimen was identical, however without the addition of Thiotepa.

Results: Toxicities were comparable, with mucositis in 7 patients (15%) in group 1 and 8 patients (17%) in group 2, (p=1.0), severe sepsis in 4 (9%) in group 1 and 3 (6%) in group 2, (p=0.7), severe venoocclusive disease in 2% of group 1 and 4% of group 2, (p=1.0) and comparable non- relapse mortality (NRM). 5-year disease free survival (DFS) median follow up of 5 years, was significantly better in group 2, 38% for group 1, and 62% in group 2, (p=0.02) and 5-year overall survival showed trend towards benefit in group 2 (62% vs 42%, p=0.06), 14/30 (46%) patients in group 1 relapsed, as compared to 4/31 patients, (12%, p=0.005) in group 2, considering NRM as competing risk.

Figure 1.

Summary/Conclusions: In conclusion, the outcome of augmented regimen (DFS and OS) is superior Flu-Bu regime, mainly due to reduction in relapses, with comparable toxicities and could eventually replace Bu/Cy.

P745
PROGNOSTIC TOOLS CAN PROVIDE PERSONALIZED OUTCOMES PREDICTION AFTER ALLOGENEIC HCT IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES
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Background: Current prognostic indices for allogeneic HCT (alloHCT) 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 Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI) and Disease Risk Index (DRI); the second applying the Center for International Blood and Marrow Transplant Research (CIBMTR) One Year Survival Outcomes Calculator, which uses large-scale multicenter data reported to the CIBMTR to provide patient-specific predictions on survival 1 year after first alloHCT.

Methods: This retrospective analysis included adult recipients of first alloHCT with CD34+ selected PBSCs from 7/8 or 8/8 donors for AML, ALL, or MDS at a single center between 1/2000 and 12/2015. The Kaplan-Meier (KM) method estimated OS and RFS. The cumulative incidence method for competing risks estimated relapse and nonrelapse mortality. We evaluated univariate association between variables of interest and OS/RFS using the log-rank test. Cox regression models assessed the adjusted effect of covariates on OS/RFS. We then determined predicted 1 year OS for each patient using the CIBMTR Calculator. Patients were divided into groups based on predicted OS probability,
in intervals of 5% +/- 2% (e.g. 65 +/-2% probability of survival at 1 year). Corresponding observed 1 year OS was then estimated for each group by the KM method. A kernel smoother was used to visually display the average of observed 1 year survival estimates over the continuous range of predicted OS.

Results: 506 patients with 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 alloHCT. 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
THROMBOTIC MICROANGIOPATHY AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: IS THERE A PROTECTIVE ROLE FOR URSODEOXYCHOLIC ACID?
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Background: Thrombotic microangiopathy (TMA) after allogeneic stem cell transplantation (alloSCT) may be a severe complication associated with high mortality. Since there is no standard treatment it would be helpful to have efficacious prophylactic measures. Some data support the beneficial effect of ursodeoxycholic acid (UDA) in RIC alloSCT.

Aims: We retrospectively analysed a total of 671 patients undergoing to reduced intensity conditioning (RIC) alloSCT, comparing the occurrence of overall TMA according to the use or not of UDA.

Methods: Both uni and multivariate analysis were performed including patient and transplant related variables at the moment of transplant to analyse the risk of developing TMA.

Results: Cumulative incidence for overall TMA was 4.8 (3.4-6.6) at 1 month, 10.1 (7.9-12.5) at 100 days, and 12.7 (10.3-15.4) at 180 days (Fig. 1). On univariate analysis, TMA was more frequent in lymphoid malignancies, Fluorarabine-melphalan based conditioning, unrelated donor, mismatched donor, prophylaxis with sirolimus-tacrolimus (SRL/TKR), prior transplant and non-UDA patients. The probability of overall TMA at 180 days in UDA patients was 9.6% (95% CI: 5.9-14.3), versus 14.7% (95% CI: 11.7-18.1) in non-UDA patients. On multivariate analysis the risk factors which remained statistically significant were use of UDA (HR 0.54; 95% CI 0.38-0.77; p=0.001), non-uda prior transplant (HR 1.57; 95% CI 1.12-2.21; p=0.009) and aGVHD (HR 3.53; 95% CI 1.13-10.7; p=0.031).

Summary/Conclusions: The use of UDA decreases the risk of TMA after alloSCT regardless of type of immunophrophylaxis.
Results: Characteristics of patients are shown in Table 1. With a median follow up for patients alive of 39 months (3-222), the median estimated survival in months and the % at +1 year and +2 years was: 114 months, 70% and 62% overall survival (OS); 23 months, 57% and 49% event free survival (EFS); 6 months, 35% and 26% GRFS1; 11 months, 46% and 38% GRFS2. 147 (24%) and 218 (35%) hadn’t any event in GRFS1 and in GRFS2 respectively. In GRFS1, event incidence was: 90 (15%) for III-IV aGvHD, 170 (27%) for cGvHD, 152 (25%) for relapse and 57 (9%) for death; In GRFS2 was 90 (15%), 65 (11%), 174 (28%) and 65 (11%) respectively. Considering those patients with cGvHD as event in GRFS1, 105 of them hadn’t the event as cGvHD at the same time in GRFS2 (since they had cGvHD requiring systemic treatment but not severe cGvHD). For these patients, the alternative event in GRFS2 was: 72 without any event, 22 relapsed and 11 died. In the multivariate analysis, factors associated with better outcomes were: for GRFS1 diagnosis (p=0.04; benefit in NHL/HL/CLL p=0.02, HR 0.71; CML5% 0.53-0.95), >4 prior lines (p=0.03, HR 1.5, CML5% 1.04-2.04), early EBMT stage (p=0.001 with early as reference; intermediate p=0.002, HR 1.5, CML5% 1.2-1.9; advance p=0.001, 2.0, 1.5-2.6), in vivo T-cell depletion (p=0.02, 0.6, 0.39-0.92) and haploidential donor (p=0.04 with HLA identical as reference, no significance 1 or 2 mismatch [p=0.18], haploidential p=0.02, 0.43, 0.25-0.74). Only early EBMT disease stage maintained significance in GRFS2 (p=0.001 with early as reference; intermediate p=0.005, 1.5, 1.1-1.9; advance p=0.001, 1.9, 1.4-2.6).

Summary/Conclusions: In our study the percentage of the GRFS endpoint was similar to previously reported. Comparing both proposed definitions, the GRFS2 endpoint define a higher population of patients without any event; so that it is possible that the morbidity is misdiagnosed. The EBMT disease score was the factor with more impact in both; it is interesting to point that although the group is smaller, haploidential donor is associated with better GRFS1.

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EFFECTIVITY AND SAFETY OF DEFIBROTIDE IN THE TREATMENT OF HEPATIC VENO-OCLUSIVE DISEASE/SINUSOIDAL OBSTRUCTION SYNDROME FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION: FINAL SUBGROUP RESULTS

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Background: Hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) is a potentially life-threatening complication of conditioning regimens for hematopoietic stem cell transplant (HSCT) and may also occur following chemotherapy without HSCT. VOD/SOS with multi-organ dysfunction (MOD) may be associated with >80% mortality. Diagnosis has traditionally been based on the Baltimore criteria or modified Seattle criteria. Defibrotide is approved for treating severe hepatic VOD/SOS post-HSCT in the European Union and for treatment of hepatic VOD/SOS with renal/pulmonary dysfunction post-HSCT in the United States. The defibrotide expanded-access protocol was designed to provide access to defibrotide prior to its approval in the United States and to collect additional data on safety and efficacy in a broader patient population, including those with and without MOD, and following HSCT or chemotherapy without HSCT.

Aims: This is an analysis of defibrotide efficacy and safety in the subgroup of patients developing VOD/SOS following HSCT, using final data from the expanded-access protocol.

Methods: The original expanded-access protocol required VOD/SOS diagnosis by either criteria or biopsy post-HSCT, with evidence of MOD (27%) or pulmonary dysfunction). The study was amended to also include patients without MOD (off-label), with VOD/SOS per modified Seattle criteria, and/or with VOD/SOS following chemotherapy without HSCT (off-label). After patients provided informed consent, defibrotide treatment (25mg/kg/d in 4 divided doses of 6.25mg/kg) was recommended ≥21 days.

Results: This analysis of final data is based on 1000 patients enrolled from 2007–2016 who had confirmed VOD/SOS following HSCT and had received ≥1 dose of defibrotide. Of these patients, 512 (51.2%) had MOD. The median age was 14 years (range 0.10–77.0), with 570 patients (57.0%) aged ≤16 years, (29.3%) <1 year, (19.3%) >16 years; 430 patients (43.0%) had MOD. Among pediatric patients, 28.2% were aged <1–23 months, 52.5% aged 2–11 years, and 19.3% aged 12–16 years. Primary diseases in ≥10% of the overall HSCT group were acute lymphocytic leukemia (19.8%), acute myelogenous leukemia (26.1%), and neuroblastoma (10.5%). Kaplan-Meier estimated Day+100 survival was 58.8% (95% confidence interval [CI], 55.7%–61.9%) in the overall HSCT group (Figure), with rates of 49.5% (95% CI, 45.0%–53.8%) in patients with MOD and 68.9% (95% CI, 64.5%–72.9%) in patients without MOD. In patients aged ≥16 years, Kaplan-Meier estimated Day+100 survival was 67.9% (95% CI, 63.8%–71.6%) and 47.1% (95% CI, 42.3%–51.8%) in patients aged >16 years (Figure). In the overall HSCT population, 210 patients (21.0%) had ≥1 treatment-related adverse event (TRAE). TRAEs occurring in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypotension (2.0%).

Figure 1. Summary/Conclusions: This final analysis of the defibrotide expanded-access protocol demonstrates favorable Day +100 survival (58.9%) in patients with confirmed VOD/SOS following HSCT, and 49.5% in those with MOD, a complication typically associated with dismal outcomes. Survival and safety findings, consistent with prior clinical trials, provide supportive evidence for the clinical utility of defibrotide for treatment of VOD/SOS in patients with and without MOD.

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GENERATION OF IMMORTAL MURINE HEMATOPOIETIC STEM/PROGENITOR CELL LINES FROM TRANSGENIC MICE
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Background: Research on hematopoietic and leukemic stem cells (LSCs) is currently limited as these cells are infrequent and their immortalization is hardly achievable. Aim: We aimed to establish a long term ex vivo culture system that allows maintenance and expansion of LSK (lin- Sca-1-, c-kit+) cells.

Methods: We adapted a technique described by the L. Carlsson lab and transduced high-purity sorted murine LSKs with Lhx2, a LIM-homeobox transcription factor, which has been reported to facilitate ex vivo expansion of immature hematopoietic cells.

Results: Lhx2 expressing hematopoietic progenitor cell (HPC(LS)) lines require SCF (stem cell factor) and IL-6 and they can be maintained in a feeder-independent culture for more than 6 months. They preserve LSK markers despite continuous proliferation. HPC(LS) cells repopulate lethally irradiated mice and re-feeding the T cell-depleted secondary cell pool (H2Kd) long-term 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/ABLp210, MLL-AF9,NrasG12D or Flt3-ITD; NrasG12D. These LSC lines show a broad multilineage potential and are shown to be radio-resistant. Nevertheless, the functional effects of irradiation on BM-MSC have not been extensively explored.

Aims: 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 were exposed to different doses of irradiation. Then, one aliquot was gamma-irradiated with a single dose of 2,5Gy whereas non-irradiated MSC from the same sample were used as controls. MSC were characterized following ISCT criteria (flow cytometry and in vitro differentiation stainings). Apoptosis was evaluated by flow cytometry using annexin-V/7AAD staining. Expression microarrays of irradiated and control-MSC were performed using Human Gene 2.0 ST Array platform (Affymetrix). RT-PCR of key genes involved in the hematopoietic supporting capacity as well as in the differentiation of MSC into osteoblasts and adipocytes was performed in both experimental groups. Finally, long term BM cultures (LT-BMC) were performed as functional assays to test the hematopoietic-supporting ability of irradiated and non-irradiated MSC. For this purpose, LT-BMC from the two experimental groups were cultured on irradiated-MSC, which 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 adipo genesis was impaired. In this regard, irradiated-MSC had significantly higher SPP1 expression (involved in late osteogenic differentiation) and lower CBP and PPAR-gamma (both genes involved in adipogenesis) compared to control MSC. After inducing in vitro differentiation, there were no differences in ALP and Alizarin Red staining but the number of adipocytes per field at days 7, 14 and 21 was significantly lower in irradiated MSC (p=0,018, p=0,046 and p=0,018, respectively). In addition, angiopoietin and SDF-1, key genes implicated in maintenance of hematopoiesis, were significantly overexpressed in irradiated-MSC (p=0,043 and p=0,028, respectively). Finally, in the functional evaluation of the hematopoietic-supporting ability of MSC by LT-BMC, we observed that the number of Cfu-GM colonies generated by the culture was significantly higher in the irradiated group after 4 and 5 weeks (p=0,046 and p=0,018, respectively) compared to the non-irradiated group. Furthermore, the number of adipocytes per field was significantly reduced in the LT-BMC.

Summary/Conclusions: Irradiation of MSC with 2,5Gy improves their hematopoietic supporting ability and modifies their differentiation capacity, increasing the osteogenesis and decreasing the adipogenesis.

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INHIBITING BCL2 AND NK CELLS IMPROVES STEM CELL TRANSPLANT OUTCOMES.
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Background: Allogeneic haematopoietic stem cell transplantation (alloHSCT) is the most effective means of preventing relapse of blood cancers, in particular AML. The curative potential of alloHSCT is largely due to the immune mediated graft-versus-leukemia (GVL) effect, which in turn is dependent on donor immunity. The dual challenge of alloHSCT is therefore to allow sufficient donor engraftment for haematopoietic and immunological reconstitution that drives the GVL effect while limiting the toxicity of conditioning and the onset of graft-versus-host disease (GVHD).

Aims: Optimize the use of BCL2 inhibitors to modify recipient NK cell function in models of alloHSCT in order to minimize GVHD severity and onset.

Hypothesis: Therapeutic targeting of recipient NK cell frequency or function pre-transplant will allow reduced intensity conditioning (RIC) and promote both donor T cell engraftment and GVHD whilst reducing the risks of GVHD.

Methods: We used a MHC-mismatched mouse model of alloHSCT, where donor and recipient cells are syngeneic cell pool (H2Kd) long-term 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/ABLp210, MLL-AF9,NrasG12D or Flt3-ITD; NrasG12D. These LSC lines show a broad multilineage potential and are shown to be radio-resistant. Nevertheless, the functional effects of irradiation on BM-MSC have not been extensively explored.

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Methods: Ten BM samples were obtained from healthy donors after informed consent. MSC were obtained and characterized following standard procedures and were exposed to different doses of irradiation. Then, one aliquot was gamma-irradiated with a single dose of 2,5Gy whereas non-irradiated MSC from the same sample were used as controls. MSC were characterized following ISCT criteria (flow cytometry and in vitro differentiation stainings). Apoptosis was evaluated by flow cytometry using annexin-V/7AAD staining. Expression microarrays of irradiated and control-MSC were performed using Human Gene 2.0 ST Array platform (Affymetrix). RT-PCR of key genes involved in the hematopoietic supporting capacity as well as in the differentiation of MSC into osteoblasts and adipocytes was performed in both experimental groups. Finally, long term BM cultures (LT-BMC) were performed as functional assays to test the hematopoietic-supporting ability of irradiated and non-irradiated MSC. For this purpose, LT-BMC from the two experimental groups were cultured on irradiated-MSC, which 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.

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Summary/Conclusions: Irradiation of MSC with 2,5Gy 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.
Background: Prolonged isolated thrombocytopenia (PT), is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT) and defined as the engraftment of all peripheral blood cell lines other than a platelet (PLT) count ≤2×10^10/L or dependence on PLT transfusions for more than 60 days after allo-HSCT. Several clinical risk factors have been proposed to be associated with PT after allo-HSCT. However, the underlying mechanisms remain to be elucidated. Emerging evidence from mouse studies has suggested that effective hematopoiesis depends on a particular bone marrow (BM) microenvironment in which hematopoietic stem cells reside. MSCs represent a key cellular component of the BM microenvironment, which are potential progenitors for osteoblasts, adipocytes, chondrocytes, and marrow stromal cells. Thus, the mechanisms of megakaryocyte and thrombocytogenesis result from the interactions between hematopoietic progenitor cells, cytokines, and marrow stromal cells derived from MSCs or MSCs 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 evaluate 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 allotransplantation, and transplant donors as normal controls (N=10). BM MSCs were cultured as previously reported. All experiments were carried out using BM MSCs derived from passages 2–4. The number and functions of BM MSCs were evaluated by flow cytometry. The expression of pro-apoptotic proteins. In addition, the expression ratio of BIM or BAX to pro-apoptotic proteins. In addition, the expression ratio of BIM or BAX to pro-apoptotic proteins. In addition, the expression ratio of BIM or BAX to pro-apoptotic proteins.

Results: Human BM MSCs were depicted 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 spindle-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 flatter 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 SA-β-positive cells, were identified in BM MSCs from PT patients. Intracellular levels of ROS and pro-p38 were evaluated in BM MSCs by flow cytometry.

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 number of in vitro-generated Th9 cells mediates GVHD. Furthermore, we analyzed a new approach to enhancing the number and function of BM MSCs derived from patients with PT has never been reported. Moreover, approaches for improving the dysfunction of BM MSCs in patients with PT are lacking.

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GRAFT-VERSUS HOST DISEASE (GVHD) DEVELOPMENT AFTER BONE MARROW TRANSPANTATION 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 involved 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 to 6–8 weeks old, NOD/SCID mice. We transplanted syngeneic BM and splenocytes from Th9 T cells and B6.SJL mice to NOD/SCID mice. We transplanted syngeneic BM and splenocytes from Th9 T cells and B6.SJL mice to NOD/SCID mice.

Results: In both MHC mismatched models used, the transplantation of allogeneic spleen cells and BM leads to GVHD characterized by a time-dependent strong increase of Th1-specific cytokines TNF-a and IFN-g in the serum of the recipient mice. Moreover, IL-9, however, was not increased in mice lacking allogeneic T cells were identified in the spleen, liver and lung of GVHD-developing animals until 29 days after transplantation, while TNF-a and IFN-g producing cells were strongly increased indicating that Th9 cells are not induced.
during GVHD. After in vitro differentiation of Th9 cells from naive T cells we obtained more than 60% of IL-9 producing cells after 5 days of culture. Th9 cells differ in their cytokine profile (IL-9+, IFN-g-, IL-13-) from Th1 and Th2 cells. Transplantation of in vitro-generated Th9 cells together with allogeneic BM cells did not induce GVHD in the MHC-disparate recipient mice, while the transplantation of unselected T cells or in vitro-generated Th1 cells induced GVHD and resulted in death in about 60% of the animals. Although no GVHD development was detected, Th9 cells migrated into lymphoid organs and GVHD target organs such as spleen and lung. Surprisingly, when the cytokine phenotype of the transplanted Th9 cells were analyzed after ex vivo isolation from spleen and liver at different time points after transplantation, the cells lost their IL-9 expression 3 days post-transfer. Furthermost, to a plasticity of Th9 cells after adoptive transfer. Systemic increase of TNF-a and IFN-g 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-a and IFN-g without strong systemic increase in these cytokines. Since TNF-a and IFN-g are cytokines associated with an anti-tumor cytotoxicity and Th9 cells are known to eliminate solid tumors, future experiments will define whether in vitro-generated Th9 cells can be used as a cellular therapy for anti-tumor responses in BM-transplanted hosts.

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**IMPROVED HSC ENGRAFTMENT IN A MOUSE MODEL OF HEMATOPOIETIC STEM CELL GENE THERAPY MEDIATED BY MSCS**

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**STEM CELL GENE THERAPY MEDIATED BY MSCS**

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ments will define, whether in vitro-generated Th9 cells can be used as a cellular therapy for anti-tumor responses in BM-transplanted hosts.

**Methods:** Isolated CD45RA+ T cells from healthy donor’s Buffy Coats were subjected to anti-CD3 plus anti-CD28 in the presence of several cytokines to polarize towards Th1 (IL-12, INF-γ- and anti-IL-4) or Th2 (IL-4, IL-13, IL-4 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-2 for Th1 cell polarization and anti-CD25, anti-IL-10, anti-CD3 and anti-IL-4 for Th2 cell polarization. In addition, the release of cytokines (IL-2, IL-4, IL-6, IL-10, TNF-a and IFN-g) in cell culture supernatants were measured by BD Human Th1/Th2 Cytokine CBA 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 INF-γ and IL-2 as determined by flow cytometry in Th1 cell culture conditions. By contrast, in the presence of IL-4 and IL-10, we observed downregulation of Th1- and Th2-promoting cytokines, respectively. In Th1 and 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 Tbet as assessed by western-blots in naive CD45RA+ cells activated with anti-CD3 plus anti-CD28 and supplemented with IL-12, INF-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 naive CD45RA+ cells exposed to pomalidomide.

**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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**EFFECT OF POMALIDOMIDE ON T CELL POLARIZATION IS MEDIATED THROUGH EPIGENETIC MODIFICATIONS.**

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**Background:** Human chronic graft-versus-host disease (CGVHD) shares clinical characteristics with a murine scleeroderma-like dermal 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 of 10^5 cells/mouse on days 3, 5 and 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 phos- phatase and tensin homolog (PTEN), phosphorylated Smad-2/3 and matrix metalloproteinase-1 (MMP-1), were observed in skin tissue. MSCs neither migrate to the skin nor affect the in vivo expansion of immune effector cells, but inhibited their infiltration into skin via downregulation of CCR4 and CCR8 expression. Ad-MSCs infused SCID-Beige mice on CD11b+ monocytes/macrophages. MSCs diminished expression of chemokines such as CCL1, CCL3, CCL8, CCL17, and CCL22 in skin. MSCs were also dependent on stimulated spleno- cytes to suppress fibroblast proliferation.

**Summary/Conclusions:** Our findings indicate that MSCs ameliorate the cutaneous GVHD immune cell migration and downregulating chemokines and chemokine receptors.

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**C57BL/6 SUBSTRAINS SHOW DIFFERENCES IN HEMATOPOIETIC RESPONSE TO ALLOGENEIC SCT AND ION**

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**Background:** C57BL/6 mice are one of the most studied in-bred mouse strains.
Although C57BL/6N (N) and C57BL/6J (J) mice are derived from the same parental C57BL/6 strain, there are key genotypic and phenotypic differences between these sub-strains. However, more than 58% of studies published involving C57BL/6 mice do not indicate the specific sub-strain employed. J mice have a five-exon deletion in the Nicotinamide nucleotide transhydrogenase (Nnt) gene that results in a non-functional protein. NNT is involved in the resolution of oxidative stress in the mitochondria. Hematopoietic stem cells (HSCs) can reconstitute the entire hematopoietic system after transplantation into hosts whose hematopoietic compartment has been ablated. This is clinically exploited as HSCs transplantation (HSCT) to treat hematologic diseases and represents the only curative therapy for many disorders. During HSCT, HSCs 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 cell (HSPC) function, here we thoroughly interrogated the frequency and function of HSPCs in J and N bone marrow (BM).

Methods: N and J peripheral blood (PB) and BM (n=9) was interrogated by flow cytometry for the absolute frequencies of all major hematopoietic lineages and HSPC compartments, respectively. 5000 J or N CD45.2 HSPCs (Lin-Sca-1+c-Kit+ cells) were transplanted along with 5000 competitor CD45.1 HSPCs into lethally irradiated mice to test for competitive in vivo hematopoietic repopulating activity and ROS levels post-transplant. 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-, MPP4: 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 lineage after transplant. J-MPP3s and J-MPP4s displayed less in vivo repopulating activity than N-MPP3s and N-MPP4s. It is known that pI:pC treatment increases ROS levels in HSPCs. We found about two-fold higher ROS levels in HSPCs isolated from pI:pC treated J mice than N mice with the exception of the myeloid progenitor compartments (CMP, GMP and MEO). J-HSPCs also generated fewer and smaller CFU than N-HSPCs when isolated from pI:pC treated mice. These data indicate that J-HSPCs cannot resolve oxidative stress as efficiently as N-HSPCs, which may be due to lower self-renewal potential after exposure to oxidative stress. Short-term J-lymphoid-biased progenitors (e.g. MPPs and CLPs) were especially sensitive to increasing ROS, which very likely drives the short-term loss of in vivo repopulating activity.

Summary/Conclusions: Based on these data, we hypothesize that loss of the Nnt gene in C57Bl/6J mice sensitizes HSPCs to oxidative stress, which compromises their short-term in vivo hematopoietic repopulating activity.

Thrombosis disorders

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GWAS RESULTS IN RED BLOOD CELL PHENOTYPES AND THEIR RELATIONSHIP WITH THROMBOSIS


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Background: Venous thromboembolism (VTE) is a complex and multifactorial disease with a estimated heritability of 60%. Intermediate phenotypes of VTE have been used to identify genetic risk factors. We previously reported a genetic correlation of 5 erythrocyte phenotypes with VTE1.

Aims: To identify single nucleotide polymorphisms (SNPs) influencing the phenotypic variance of erythrocyte parameters, especially those related to VTE, in Spanish families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT2) Project.

Methods: Genome-wide association analyses (GWAS) with ~10M SNPs were performed for eighteen erythrocyte phenotypes in 935 subjects belonging to 35 extended families with thrombosis of GAIT2. The erythrocyte phenotypes evaluated were: Hemoglobin (Hb), red blood cell count (RBC), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), reticulocyte (RET), low fluorescence reticulocyte (LFR), middle fluorescence reticulocyte (MFR), high fluorescence reticulocyte (HFR), reticulocyte fluorescence index (IRF), haptoglobin (HP), serum iron (Fe), total iron binding capacity (TIBC), saturation index (SI), serum ferritin (FT) and serum transferrin receptor (TFR).

Results: We identified 12 SNPs showing association with the 5 erythrocyte phenotypes previously related to VTE (Table 1). Interestingly, the rs56036145 that showed association with TFR is an intronic variant located in the gene tissue factor pathway inhibitor 2 (TFPI2), which encodes a protein that inhibits a variety of serine proteases of blood coagulation, such as activated factor VII (FVIIa/TF), FXa, plasmin and plasma kallikrein. These data reinforce our previous report of genetic correlation of TFR with VTE. The most significant SNP-associations were reported.

Table 1. Top SNP-associations with erythrocyte phenotypes related to VTE from GWAS in GAIT2.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>SNP</th>
<th>Chr</th>
<th>Type</th>
<th>Closest gene</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>rs4497451</td>
<td>11</td>
<td>APOE</td>
<td>ANGK1, LIPA</td>
<td>1.6×10⁻³⁷</td>
</tr>
<tr>
<td>MDW</td>
<td>rs1501261</td>
<td>19</td>
<td>APOE</td>
<td>ANGK1</td>
<td>1.5×10⁻³⁷</td>
</tr>
<tr>
<td>MCV</td>
<td>rs11194609</td>
<td>100</td>
<td></td>
<td>COLA1A</td>
<td>1.6×10⁻⁶⁷</td>
</tr>
<tr>
<td>MCH</td>
<td>rs4565971</td>
<td>19</td>
<td>APOE</td>
<td>ANGK1, LIPA</td>
<td>2.9×10⁻³⁷</td>
</tr>
<tr>
<td>MCHC</td>
<td>rs772470</td>
<td>19</td>
<td>APOE</td>
<td>ANGK1, LIPA</td>
<td>6.4×10⁻³⁷</td>
</tr>
<tr>
<td>MCV</td>
<td>rs600215</td>
<td>19</td>
<td></td>
<td>MTHFD2</td>
<td>8.6×10⁻⁷</td>
</tr>
<tr>
<td>MCHC</td>
<td>rs927102</td>
<td>19</td>
<td></td>
<td>MTHFD2</td>
<td>2.1×10⁻⁷</td>
</tr>
<tr>
<td>MCV</td>
<td>rs529701</td>
<td>19</td>
<td></td>
<td>MTHFD2</td>
<td>4.8×10⁻⁷</td>
</tr>
<tr>
<td>MCHC</td>
<td>rs529701</td>
<td>19</td>
<td></td>
<td>MTHFD2</td>
<td>8.6×10⁻⁷</td>
</tr>
<tr>
<td>MCV</td>
<td>rs529701</td>
<td>19</td>
<td></td>
<td>MTHFD2</td>
<td>2.1×10⁻⁷</td>
</tr>
<tr>
<td>MCHC</td>
<td>rs529701</td>
<td>19</td>
<td></td>
<td>MTHFD2</td>
<td>4.8×10⁻⁷</td>
</tr>
<tr>
<td>TFR</td>
<td>rs259149</td>
<td>19</td>
<td></td>
<td>MTHFD2</td>
<td>3.3×10⁻⁴</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.

Reference

This work was supported by RIC RD12/00420032, FIS PI12/00612 and FIS PI15/0269 grants

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ESSENTIAL THROMBOCYTHEMIA (ET) AND POLYCYTHEMIA VERA (PV) PATIENTS SHOW AN INCREASED THROMBUS FORMATION IN A DYNAMIC MODEL OF PLATELET ADHESION

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DOAC ASSOCIATED MAJOR GASTROINTESTINAL BLEEDING: REAL LIFE EXPERIENCE FROM A UNIVERSITY TEACHING HOSPITAL, UK

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1MacNeal Hospital, Berwyn, United States
2Department of Internal Medicine, CARIM, Maastricht University, Maastricht, Netherlands

Background: ET and PV are characterized by a high incidence of arterial and venous thrombosis. Platelet (PLT) count is not an independent risk factor for thrombosis in these conditions. However, no information is available on patient PLT thrombosis formation capacity, i.e. the PLT thrombus formation capacity in a dynamic condition.

Aims: We wanted to evaluate, in a group of ET and PV patients, the PLT thrombus formation capacity by an ex-vivo dynamic model of PLT adhesion under flow conditions, and to establish the influence of JAK2-V617F/Calreticulin (CalR)/MPL mutations, hematological parameters, and ongoing therapies.

Methods: One hundred-thirty patients, i.e. 78 ET (32 M/46 F; median age=61 years, range 28-86) and 52 PV (26 M/26 F; median age=65 years, range 38-87) were enrolled after informed consent. For the adhesion assay, peripheral venous whole blood was drawn in sodium citrate, recalciﬁed in the presence of heparin, and perfused over a collagen-coated surface for 4 min. at a shear rate of 1,000 s^-1. PLTs were then stained with an anti-CD62P (P-selectin)-FITC antibody to detect PLT activation, and annexin-V-AlexaFluoro67 to detect pro-coagulant phosphatidylserine expression. After staining, phase contrast and fluorescence images of adherent PLTs were taken in random fields using an EVOS® microscope. Results are expressed as the means±SEM of the % of area covered by all PLTs (% coverage), or as the % of adherent PLTs positive for pro-coagulant (PLTs-PS) or phosphatidylserine (PS) expression.

Results: PLT adhesion was signiﬁcantly higher (p<0.01) in both ET (45±3.1%) and PV patients (48±1.6%) compared to healthy controls (37.5±1.7%), while no difference was found between ET and PV patients. The analysis of the mutational status shows that ET PLT adhesion was highest in JAK2-V617F mutation carriers (n=4; coverage: 47.7±2.4%, p<0.001 vs controls), followed by CalR-positive patients (n=21; coverage: 45.5±3.2%, p<0.05 vs controls, p=m.s. vs JAK2-V617F), while PLT adhesion of MPL-positive (n=3; coverage: 32.1±2.1%) or triple negative (n=13; coverage: 42.6±2.5%) ET patients was not statistically different from controls. In PV, no statistically significant difference was observed between subjects with >50% vs those with <50% JAK2-V617F allele burden. According to treatment, we observed that ET patients treated with the combination of aspirin+hydroxyurea presented the lowest PLT adhesion, while in PV no signiﬁcant difference was observed between different anti-coagulant regimens. PLT count correlated (p<0.01) with PLT adhesion only in CalR-positive ET patients. The analysis of adherent PLT surface markers shows no difference in P-selectin expression between whole patients and controls. Differently, phosphatidylserine expression was signiﬁcantly reduced (p<0.01) in both ET and PV compared to healthy subjects.

Summary/Conclusions: ET and PV platelets show an increased PLT thrombus formation potential, particularly in patients carrying the JAK2-V617F mutation. On the basis of these results, it is worth to include a dynamic PLT adhesion assay in risk prediction models to evaluate the predictive value of thrombotic events in ET and PV patients. [Project funded by “AIRC-IG2013” grant Nr. 14505 of the “Italian Association for Cancer Research” (A.I.R.C.)].

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Abstract withdrawn.

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INCIDENCE OF VENOUS THROMBOEMBOLISM IN PATIENTS UNDERGOING LOWER LIMB SURGICAL REvascularization: IS THROMBOPROPHYLAXIS WARRANTED?

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Background: The incidence of postoperative deep vein thrombosis (DVT) or consequential pulmonary embolism (PE) in patients undergoing lower extremity surgical revascularization procedures is not well studied. The need for routine anticoagulation for DVT/PE prophylaxis after the lower limb surgical revascularization remains controversial.

Aims: The purpose of this study is to retrospectively evaluate the incidence of postoperative DVT/PE in patients undergoing lower limb surgical revascularization.

Methods: Charts for patients undergoing lower limb surgical revascularization, from 01/01/2010 to 12/31/2015, were evaluated for DVT/PE. DVT/PE within three months of the revascularization was considered to be a postoperative DVT/PE. Patients undergoing multiple procedures were counted as different cases if they were on different days. Multiple procedures on a patient on the same day were considered a single case. Patients with hypercoagulable states or previous history of DVT were excluded. Descriptive statistics and t-test was used to analyze incidence of DVT/PE and assess the importance of postoperative thromboprophylaxis.

Table 1.

<table>
<thead>
<tr>
<th>Procedure performed</th>
<th>DVT/PE within three months from surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>笔者</td>
<td></td>
</tr>
<tr>
<td>Ballon angioplasty</td>
<td>15</td>
</tr>
<tr>
<td>Bypass aorta-femoral</td>
<td>18</td>
</tr>
<tr>
<td>Bypass femoral-peroneal</td>
<td>3</td>
</tr>
<tr>
<td>Bypass femoral-femoral</td>
<td>12</td>
</tr>
<tr>
<td>Bypass femoral-polipleve</td>
<td>252</td>
</tr>
<tr>
<td>Bypass femoral-tibial</td>
<td>6</td>
</tr>
<tr>
<td>Lower limb embolectomy</td>
<td>3</td>
</tr>
<tr>
<td>Femoral artery exploration</td>
<td>39</td>
</tr>
<tr>
<td>Thrombectomy</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>354</td>
</tr>
</tbody>
</table>

Results: Between 1/1/2010 to 12/31/2015, 360 patients were found to have undergone lower extremity surgical revascularization. Study population included 200 males and 160 females. Mean patient age was 69.54 years. One patient had a previous history of DVT and was excluded. Overall, of the 359 patients, five (1.4%) were recognized to have a new DVT/PE within 3 months of the surgery. One patient developed DVT in the contralateral limb, and one developed it in the arm. Patients were recognized to have a new DVT/PE, on an average, at 7.6 days after the surgery. A one sided t-test demonstrated that the average
postoperative day for recognition of DVT/PE was significant greater than 3.5 (7.6 vs 3.5, \(P=0.048\)). Patients developing DVT/PE did not differ by obesity or age when compared with non-DVT/PE population.

**Summary/Conclusions:** There have been only a few studies to assess the incidence of DVT/PE in patients undergoing lower limb surgical revascularization. In our study population, 1.4% of patients had evidence of DVT/PE. This study shows a low risk of venous thromboembolism. The 2012 American College of Chest Physicians (ACCP) guidelines for prevention of venous thromboembolism in nonorthopedic surgical patients (Chest 2012; 141(2)(Suppl) e227s-e277s), requires the use of pneumatic compression devices (PCDs), over no prophylaxis, to prevent DVT/PE in low risk patients. Since, patients with lower limb surgeries are not a good candidate for PCDs, pharmacological thromboprophylaxis with low dose heparin may be warranted. Given that bleeding is a potential complication in these patients, it might be prudent to start thromboprophylaxis 3-5 days after the surgery. Further studies are needed to assess the bleeding risks of postoperative thromboprophylaxis after surgical revascularization procedures.

### Table 1. Values, heritabilities, household effect and significant covariates effects.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Value</th>
<th>b</th>
<th>p (value)</th>
<th>(\rho_e)</th>
<th>Covariates</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12 (mmol/L)</td>
<td>441.2±40.7 (74-4558)</td>
<td>0.47</td>
<td>2.95 x 10^{-5}</td>
<td>0.11</td>
<td>Age, comorbidity, smoking</td>
</tr>
<tr>
<td>SF (mmol/L)</td>
<td>2.1±0.6 (0.2-4.1)</td>
<td>0.27</td>
<td>2.3 x 10^{-1}</td>
<td>0.07</td>
<td>Sex, comorbidity, smoking</td>
</tr>
<tr>
<td>RCF (mmol/L)</td>
<td>124.9±41.3 (473-3554)</td>
<td>0.42</td>
<td>1.85 x 10^{-2}</td>
<td>0.06</td>
<td>Sex, comorbidity, smoking</td>
</tr>
<tr>
<td>HCY (mmol/L)</td>
<td>10.4±0.5 (2.7-9.7)</td>
<td>0.36</td>
<td>3.61 x 10^{-1}</td>
<td>0.41</td>
<td>Sex, comorbidity, 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 ensembles</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>B12</td>
<td>19</td>
<td>MTHFR rs1801133</td>
<td>1.7 x 10^{-1}</td>
</tr>
<tr>
<td>SF</td>
<td>7</td>
<td>MTHFR rs1801133</td>
<td>1.3 x 10^{-6}</td>
</tr>
<tr>
<td>RCF</td>
<td>19</td>
<td>TEGMC rs10478171</td>
<td>1.3 x 10^{-5}</td>
</tr>
<tr>
<td>HCY</td>
<td>1</td>
<td>MTHFR rs1801133</td>
<td>2.5 x 10^{-10}</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>Intron (rs7430416)</td>
<td>2.6 x 10^{-9}</td>
</tr>
</tbody>
</table>

Summary/Conclusions: In the GAIT2 study, genetic and environmental factors were related to B12, SF, RCF and HCY. Moreover, a relationship was observed between B12 and VTE. In the GWAS analysis some signals were previously reported (FUT2 and B12 or MTHFR with SF and HCY). New signals were found that need to be clarified, especially their possible relationship with susceptibility to thrombosis.

This work was supported by RIC RD12/0042/0032, FIS PI12/00612 and FIS PI 15/0269 grants.
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 their presence in 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).

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ARE WE TESTING APPROPRIATELY FOR THE LUPUS ANTICOAGULANT (LA)?

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Background: The diagnosis of antiphospholipid syndrome (APS) requires the presence of thrombosis or defined pregnancy morbidity in addition to the presence of antiphospholipid antibodies on at least 2 occasions. Patients should be tested for antiphospholipid antibodies if they fulfil the required clinical criteria. Lupus anticoagulant may also be tested for when investigating a prolonged activated partial thromboplastin time which does not correct on mixing studies.

Aims: The aim of our study was to examine retrospectively the frequency of lupus anticoagulant (LA) testing in our institution, which we suspected to be high, and the incidence of positive results leading to a diagnosis of APS.

Methods: A total of 914 requests for LA were received over a 5 month period between 1st of May and 30th September 2014. We examined which departments were requesting the tests and the clinical indications for testing.

Results: Over 90% (829) of LA tests were negative. Nine percent (85) of tests demonstrated a positive LA. 33 patients had experienced arterial (11) or venous (22) thrombosis. There were 3 patients who fulfilled the clinical criteria for pregnancy morbidity in APS. A total of 6 patients experienced miscarriage before 10 weeks gestation; however none of these patients had the defined 3 miscarriages. There was one preterm delivery at 25 weeks due to pre-eclampsia. A further 3 patients had a still birth, one of which had an identifiable cause. In total, of the 85 positive results, 12 patients had a confirmed diagnosis of APS; a further 25 patients had the clinical manifestations fitting the clinical criteria for APS. Forty eight patients had a positive LA but did not fit the clinical criteria for a diagnosis of APS. The clinical specialties requesting the majority of tests were obstetrics and gynaecology (231), rheumatology (179) and clinical haematology (165). The speciality requesting the majority of tests for a diagnosis of APS. The clinical specialities requesting the majority of tests were obstetrics and gynaecology (231), rheumatology (179) and clinical haematology (165).

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 their presence in 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).

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RESULTS OF USING BRIDGING THERAPY WITH SODIUM BEMPARIN AT THERAPEUTIC-DOSE

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Background: Bridging therapy consists of the administration of a fast-acting anticoagulant such as the low-molecular-weight heparin (LMWH) during the period of cessation of oral anticoagulant therapy. The decision to continue with anticoagulant therapy or to discontinue the treatment with the establishment of the Bridging therapy have been carried out carefully and on an individual basis. While taking this decision, we have taken into account three factors: the urgency of surgery or invasive process, the risk of bleeding and thrombotic risk for the patient. In recent decades, there have been multiple studies supporting the LMWH treatment, at least as safe and effective and more cost-beneficial than unfractionated heparin (UFH) in the 6-12 days of venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). Therefore, the LMWH is considered as the drugs of choice in the prevention of venous thromboembolism.

There are several types of commercialized LMWH, with different pharmacological properties, such as molecular weight, anti-Xa/IIa ratio and average life. The sodium bemparin is the LMWH with greater anti-Xa/IIa ratio, which implies a lower risk of bleeding. In addition, it has shown a low incidence of VTE and bleeding in actual clinical practice.

Aims: There are few published data from bridging therapy at therapeutic doses in patients treated with oral anticoagulants (AVK) and perioperative management. It is intended to assess the efficacy (recurrence of thrombosis) and safe use of sodium bemparin at anticoagulant doses on the bridging therapy and possible thrombotic and/or hemorrhagic complications (major and minor bleeding) resulting from this use.

Methods: We have analyzed 975 bridging therapies at full dose in our clinic in the last year. They were made to a total of 650 patients (315 men and 335 women) with CHADS/VASC ≥2, aged between 15 and 92, with an average age of 69 years old. The reasons of anticoagulation in our patients were atrial fibrillation, mechanical prostheses, DVT, pulmonary embolism and recurrent thrombosis in patients with thrombophilia. In 70% of the cases, there were comorbidities, such as heart failure, chronic obstructive pulmonary disease, anemia, kidney failure, liver disease and long-term aftereffects of stroke. The bridging therapy has consisted of suspending treatment (with or without anticoagulant) to 6 days (warfarin) before the procedure, and replacing it by sodium bemparin at full doses <50 kg: 5.000 IU/24h, 50 to 70 kg: 7.500 IU/24 h, 70-100 kg: 10.000 IU/24 h and >100 kg: 12.500 IU/24 h, and administration of a prophylactic dose of 3.500 IU, 12 hours before the procedure, and another dose 6-12 hours after the procedure, depending on the risk of bleeding of the intervention and the thrombotic risk of the patient’s disease. The bridging therapy has been performed in 225 cases of major surgery (orthopedic surgery, ophthalmological procedures, valvular replacements etc), 340 cases of minor surgery (removal of nevus, complex dental extractions, dental implants), 295 cases of invasive procedures (colonoscopies, endoscopies...), 50 cases of bleeding caused by AVK (epistaxis, petechiae and bruises, hemoptysis, menorrhagia and gastrointestinal bleeding), 30 cases of hospitalization with INR decompensation with various causes (infectious endocarditis, pneumonia, uncompensated heart failure...) and 35 cases for thrombophilia study.

Results: As complications of using bemparin sodium, there have been: 40 cases of hematomas at the needle puncture sites. There was neither cases of major bleeding nor cases of thrombosis.

Table 1.

Summary/Conclusions: Sodium bemparin administered at therapeutic doses (115 IU/kg/24h) in the perioperative period, according to the scheme described above, it is associated with a low incidence of recurrence of VTE and bleeding. The complications presented in our sample have been very few, in patients with associated co-morbidities. In our study, sodium bemparin has shown to be safe and effective with minimal bleeding complications. Treatment should be administered on an individual basis according to each patient and factors related to surgery. Further studies will confirm our results.
Targeted therapies in relapsed in chronic lymphocytic leukemia

IBRUTINIB IN PREVIOUSLY TREATED CHRONIC LYMPHOCYTIC LEUKEMIA: UPDATED EFFICACY AND SAFETY OF THE RESONATE STUDY WITH UP TO FOUR YEARS OF FOLLOW-UP


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Background: Ibrutinib is a first-in-class, once-daily oral inhibitor of Bruton’s tyrosine kinase. Ibrutinib as a single agent is indicated by the EMEA and US FDA for the treatment of adult patients with CLL and allows for treatment without chemotherapy. The phase 3 RESONATE trial in patients with relapsed CLL showed superior efficacy of ibrutinib compared with ofatumumab (Byrd NEJM 2014). Aims: We report updated safety and efficacy results of the RESONATE trial with up to 4 years of follow-up. Methods: Eligibility criteria included ≥1 prior therapy, ineffectiveness for treatment with a purine analog, and ECOG performance status 0-1. Informed consent was obtained from all patients prior to study initiation. Patients received oral ibrutinib (420 mg once daily) until disease progression or unacceptable toxicity or intravenous ofatumumab (300 mg weekly; 2000 mg weekly for 7 weeks and then every 4 weeks for 16 weeks) for up to 24 weeks. At the interim analysis (median follow-up of 9 months), the data monitoring committee declared superior efficacy of ibrutinib vs ofatumumab for progression-free survival (PFS) and overall survival (OS), and access to ibrutinib was recommended for all patients in ofatumumab arm who had disease progression. Long-term follow-up of efficacy endpoints are open for investigator assessment. Patients randomized to ofatumumab were censored at crossover. Results: Randomized patients were randomized to receive ibrutinib (n=195) or ofatumumab (n=196). The median age was 67 years, with 40% age ≥70 years, and Rai stage III/IV in 57% of patients. At a median follow-up of 44 months (maximum 53 months) for the ibrutinib arm, PFS was significantly longer for ibrutinib vs ofatumumab (median NR vs 8 months, [HR 0.133; P<0.0001]). The 3-year PFS was 59% for ibrutinib vs 3% for ofatumumab. A significant PFS benefit was observed across baseline subgroups. In the ibrutinib arm, PFS for the del11q subgroup trended to have the most favorable outcome; however, PFS outcomes were not statistically different for patients with del17p or del11q or patients without these FISH abnormalities. At time of analysis, with the majority of patients randomized to ofatumumab (68%) crossing over to receive ibrutinib therapy, OS was longer for ibrutinib vs ofatumumab (median OS NR for either arm). The 3-year OS rate for ibrutinib was 74%. The ORR for ibrutinib was 91% with a CR/CRi rate that increased over time (currently 9%). Baseline cytopenias improved with extended ibrutinib therapy for hemoglobin (85% of patients), platelet (95% of patients), and absolute neutrophil counts (95% of patients). The adverse event (AE) profile of ibrutinib was consistent with previous reports. During a follow-up of up to 4 years, major hemorrhage occurred in 6%, grade ≥3 atrial fibrillation occurred in 6%, and grade ≥3 hypertension occurred in 8% of patients. The incidence of most ≥3 AEs decreased from year 1 vs year 2-3: neutropenia: 18% vs 8%; pneumonia: 11% vs 4%; atrial fibrillation (AF); and respectively. The most frequent reasons for treatment discontinuation were progressive disease (27%) and AEs (12%). At analysis, 90 patients randomized to ibrutinib (46%) continue to receive ibrutinib.

Summary/Conclusions: In this international phase 3 RESONATE study with median follow-up of up to 4 years, long-term treatment with ibrutinib showed a favorable tolerability profile with sustained PFS and OS benefit regardless of high-risk cytogenetics. The results in relapsed del17p and del11q patients compared favorably to those previously reported in phase 2 studies.

S771 VENETOCLAX IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA WITH 17P DELETION: OUTCOME AND MINIMAL RESIDUAL DISEASE FROM THE FULL POPULATION OF THE PIVOTAL M13-892 TRIAL


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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.

Aims: This phase 1 trial evaluates the safety/efficacy of the triplet combination of venetoclax (V), UBLITUXIMAB (UTX), and TGR-1202 in pts with previously untreated or previously treated advanced CLL. METHODS: The combination of UTX, TGR-1202, and ibrutinib is a novel anti-CD20 mAb+PI3Kδ+BTK inhibitor (ibrutinib) in pts with B-cell malignancies. This is an open-label study with 28-day cycles. Twice daily (BID; 30 mg/m² BID x 5) of UTX and ibrutinib dosed at 420 mg (CLL) or 560 mg (NHL), both on C1D1. Dose escalation: twice daily 30 mg/m² BID x 5 followed by twice daily 60 mg/m² BID x 5 (24 mg/m² QD), and 80 mg/m² BID x 5 (32 mg/m² QD). RESULTS: 101 pts (N=101) were evaluable for efficacy; 75% had del(17p), and 32% were fludarabine refractory. 38% of 2 prior therapies (range, 0–6); 32% were fludarabine refractory; 11% had previously received a B-cell receptor signaling inhibitor (BCRi); 48% had nodes ≥5 cm; and 78% had unmutated IGVH. The median duration of venetoclax therapy was 16.7 months (range 0.02–43.1 months). Primary reasons for discontinuation were toxicity (32%), adverse events (AEs) (20%), withdrawal of consent (8%), and other reasons (10%). 24-month estimates for progression-free survival (PFS) and overall survival (OS) were 78.5% and 120% respectively. 3-24 mos). 53% of evaluable CLL pts had high-risk cytogenetics and 4/6 DLBCL pts were non-GCB. 1 CLL pt (17p/11q del) ref to PI3Kδ and ibrutinib achieved a CR. Of 24 pts on study (10-27 mos). Med DOR was not reached (range 3-24 mos).

Response to UTX+TGR-1202+ibrutinib for advanced CLL

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0.02%). Pts observed. Two grade 3 dose limiting toxicities were observed at 45 mg BID (one each of grade 3 thrombocytopenia, pyrexia, rash, infusion reaction, ischemia (each at 29%), thrombocytopenia, cough (each at 26%), anemia (24%) and sinusitis (21%). OR 3/4 AE’s (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>
</thead>
<tbody>
<tr>
<td>CLL/SLL</td>
<td>19</td>
<td>3</td>
<td>16</td>
<td>84%</td>
</tr>
<tr>
<td>FL/MZL</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>86%</td>
</tr>
<tr>
<td>DLBCL</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>MCL</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>75%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This is the first known triplet combination of an anti-CD20 mAb+PI3Kδ+BTK inhibitor. The combination of UTX, TGR-1202, and ibrutinib has been well tolerated with activity observed across heavily pre-treated and therapy-resistant B-cell malignancies. Expansion cohorts at the highest dose (800mg TGR-1202+full dose ibrutinib) are underway. Future trials for the triplet are warranted.

S773

THE DUAL SYK/JAK INHIBITOR CERDULATINIB DEMONSTRATES COMPLETE INHIBITION OF SYK AND JAK AND RAPID TUMOR RESPONSES IN A PHASE 2 STUDY IN PATIENTS WITH RELAPSED/REFRACTORY B CELL MALIGNANCIES

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Background: Subsets of B cell malignancies are addicted to B cell antigen receptor (BCR) signaling for survival. Co-stimulation of the BCR with IL-2 or IL-4 in normal B cells significantly enhances cellular activation relative to BCR or cytokine stimulation alone, and combining SYK selective and JAK selective inhibitors synergize to suppress this response (Coffey et al., 2013). Hence, BCR/SYK and cytokine JAK/STAT signals cooperate to control B cell activation. This cooperation appears to be relevant to B cell malignancies as well. IL-4 promotes the survival of CLL cells in culture via up-regulation of MCL1 and BCL-XL, protecting the tumor from death induced by fludarabine and chlorambucil (Steele et al., 2010) and by idelalisib and ibrutinib (Aguilar-Hernandez et al., 2016). Also, unlike ibrutinib, combined SYK and JAK inhibition by cerdulatinib induces apoptosis in primary CLL cells and leads to down-regulation of MCL1 and BCL-XL (Blunt et al., 2015) and induces apoptosis in cells from ibrutinib-resistant CLL patients (Guo et al., 2017). It also induces apoptosis in primary DLBCL and DLBCL cell lines that carry BCR pathway mutations resistant to ibrutinib (Ma et al., 2015). Clinical trials suggest that SYK/JAK inhibition may therefore represent a powerful strategy to control B cell malignancies. A phase 1 dose escalation study of cerdulatinib in 43 patients with relapsed/refractory CLL and NHL was recently completed (Hamlin et al., EHA Congress 2016). Inhibition of both BCR/SYK and JAK/STAT signaling pathways by >90% in peripheral blood mononuclear cells (PBMCs) and CLL cell lines correlates with clinical improvement in CLL and FL, including in patients who had relapsed on prior BCR inhibitor therapy. No consistent hematopoietic toxicity, anemia, thrombocytopenia or neutropenia was observed. Two grade 3 dose limiting toxicities were observed at 45 mg BID (fatigue, pancreatitis). 35 mg BID was identified as the Phase 2 dose based on Phase 1 data and on PK/PD modeling.

Aims: The primary aim of the study was to understand the safety and activity of cerdulatinib in B-cell malignancies.

Methods: This is an open-label study with 28-day cycles. Twice daily (BID; 30
mg and 35 mg) dosing was evaluated. Pharmacokinetics (PK), pharmacodynamics (PD), and safety were monitored, as well as an assessment of efficacy. Clinical response was assessed by standard criteria. Potency and specificity for SYK and JAK pathway inhibition were measured in whole blood assays by monitoring signaling responses following ligation of the BCR and receptors for IL-4. Serum markers of inflammation, minimal residual disease (MRD) and apoptosis in CLL patients were also measured.

Results: A phase 2 study was initiated in May 2016 to enroll up to 40 patients in each of the three cohorts; 1) relapsed/refractory CLL/SLL, 2) relapsed/refractory indolent NHL, and 3) relapsed DLBCL, MCL and transformed FL. As of March 1, 2017, 37 patients have been enrolled, 17 with CLL/SLL, 15 with indolent NHL (10 FL, 4 MZL, 1 WM), and 5 with aggressive NHL (3 DLBCL, 1 MCL, 1 tFL). Median patient age is 70 years (range, 51-93). The median number of prior therapies is 3 (range 1–7). 11 patients had prior BTK or PI3K inhibitor therapy. The safety profile has been similar to what was seen in the Phase 1 study. However, 3 patients at 35 mg BID achieved higher than expected drug concentrations and had SAEs (2 grade 5 infections, 1 grade 3 pancreatitis). The starting dose was reduced to 30 mg BID and a PK monitoring and dose reduction strategy has been implemented. To date, this has resulted in a better safety profile without PK outliers. The most common AEs of any grade have been diarrhea (27%), fatigue (27%) and nausea (24%). Grade 3 AEs occurring in more than one patient are infection (5 patients), abdominal pain (3 patients) and hypertension (3 patients). As seen in phase 1, significant inhibition of SYK and JAK signaling pathways in peripheral blood is observed. Evidence for tumor cell mobilization to peripheral blood in CLL/SLL is consistently observed following one week of therapy. PRs have been seen in all 3 cohorts including 10 of 13 (77%) CLL/SLL, 8 of 9 (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 and 3 of 6 (50%) FL patients evaluated. Of these 13 PRs, 12 are still on drug with 4 patients in response for greater than 6 months. In addition, PRs have been seen in patients who relapsed on ibrutinib (FL patient, 8+ months) and venetoclax (SLL patient, 7+ months) therapy. As demonstrated preclinically, we have seen evidence of apoptosis (Annexin V+ B-cells) in 6 CLL patients. 5 of these patients had a PR at the end of the 2nd cycle (Figure 1).

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.

**Follicular lymphoma - Clinical**

S774

COMPARISON OF CONTRAST-ENHANCED CT-BASED RESPONSE WITH PET ASSESSMENT 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 unacceptable (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=8) scans were considered non-responders; these pts and those with PD prior to S AEs were excluded from landmark PFS analyses. At EOI, 390/655 (65.5%) pts achieved a PET-CR according to IRC, comprising 212/297 (71.4%) G-chemo pts and 178/358 (50.0%) R-chemo pts. However, for these 390 pts, evaluable CT responses were 161 CR (41.3%), 216 PR (55.4%) and 5 SD/PD (1.3%; Table 1). Conversely, PET assessment showed a PET-CR in 161/177 (91.0%) of pts achieving a CT-CR, and 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 INV and IRC 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 83.9–90.8) 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).

Table 1. CT and PET clinical response assessment by IRC at EOI

<table>
<thead>
<tr>
<th>PET, n (%)</th>
<th>CT, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>PR</td>
</tr>
<tr>
<td>CR</td>
<td>61 (27.1)</td>
</tr>
<tr>
<td>PR</td>
<td>7 (2.2)</td>
</tr>
<tr>
<td>SD</td>
<td>3 (0.9)</td>
</tr>
<tr>
<td>PD</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>NE</td>
<td>5 (0.8)</td>
</tr>
</tbody>
</table>

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
and IRC PET evaluation. Comparison of PFS based on CT-response and re-analysis of PFS scans applying the now recommended 5-point scale for PET response assessment will be presented. Pooled analyses of these data and from other studies with longer follow-up may determine PET response as a reliable early surrogate for PFS and OS, providing a platform for study of response-adapted therapy.

Figure 1.

S775
IMMUNOCHEMOTHERAPY WITH OBINUTUZUMAB OR RITUXIMAB IN PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA IN THE RANDOMIZED PHASE III GALLIUM STUDY: ANALYSIS BY CHEMOTHERAPY REGIMEN


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Background: The Phase III GALLIUM study (NCT01332968) showed that obinutuzumab (GA101; G) significantly prolonged PFS in previously untreated FL pts relative to rituximab (R) when combined with chemotherapy (chemo; CHOP, CVP or bendamustine [B]). Grade 3–5 and serious AEs were more common with G-chemo.

Aims: To explore outcomes by immunochemotherapy regimen.

Methods: Pts were aged ≥18 yrs with documented, previously untreated FL (grades 1–3a), advanced disease (stage III/IV or stage II with tumor diameter ≥7cm), ECOG PS 0–2, and requiring treatment according to GELF criteria. Chemo regimen was allocated by center. Pts were randomized 1:1 (stratified by chemo, FLIPI-1 group and geographic region) to R 375mg/m2 on day (D) 1 of each cycle (C) or G 1000mg on D1, 8 and 15 of C1 and D1 of C2–8, for 6 or 8 cycles depending on chemo. Pts continued to receive R or G every 2 months for 2 yrs or until progression. The cut-off date for this analysis was September 10 2016. All pts gave informed consent.

Results: 1202 FL pts were randomized. Baseline characteristics were generally similar across chemo groups, although B and CVP pts had relatively more comorbidities, e.g. GI and vascular disorders, than CHOP pts. After 41.1 months’ median follow-up, investigator (INV)-assessed PFS remained superior for G-chemo relative to R-chemo (HR, 0.68; 95% CI 0.54–0.87; p=0.0016) with consistent HRs across chemo groups (Figure 1). HRs for secondary time-to-event endpoints were supportive of the primary analysis. Difference in frequency of grade 3–5 AEs between arms was highest with CHOP and CVP (Table 1). Rates of second neoplasms and grade 3–5 infections were similar in G and R arms for CHOP and CVP but not for B. In all chemo groups, SAEs were more frequent with G than R, and AEs causing treatment discontinuation and fatal AEs were similar. Reductions in T-cell counts were more pronounced and prolonged in the B group than CHOP or CVP groups.

Figure 1.

Table 1. Safety summary (number (%) of FL pts* with ≥1 AE).

Summary/Conclusions: In treatment-naive FL pts, PFS was superior with G-chemo relative to R-chemo with consistent effects across chemo regimens. Some differences were seen in safety profiles between chemo regimens, but comparisons may be confounded by the lack of randomization.

S776
EFFICACY AND SAFETY OF COPANLISIB IN PATIENTS WITH RELAPSED/REFRACTORY FOLLICULAR LYMPHOMA: A SUBSET ANALYSIS OF THE CHRONOS-1 STUDY


314 | haematologica | 2017; 102(s2)
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-isofroms. 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 yrs, 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 1-105); 33 (32%) patients remained on treatment. Per investigator assessment, 87 of 96 evaluable patients (91%) had some degree of tumor shrinkage as best response, and 58/96 (60%) had >50% tumor shrinkage (Figure 1). For all patients in the phase II study, the most common treatment-emergent AEs occurring in >25% of patients included (all grade/grade 3): diastolic (34%/6%), reduced neutrophil count (30%/24%), fatigue (30%/32%), and fever (25%/4%). Hyperglycemia (50%/41%) and hypertension (30%/24%) were transient. The incidence of pneumonitis (8%/1.4%), hepatic enzymopathy (AST 28%/1.4%; ALT 23%/1.4%), opportunistic infection (1.4%) and colitis (0.7%) were low. Six deaths were observed, 3 of which were attributed to copanlisib: one lung infection, one respiratory failure, and one thromboembolic event.

Figure 1.

Summary/Conclusions: Copanlisib was highly active as a single agent in heavily pretreated relapsed/refractory FL patients and resulted in responses in the majority of patients with a median duration of response of 370 days and >50% tumor shrinkage as best response, and 58/96 (60%) had >50% tumor shrinkage. Toxicities were manageable, with a low incidence of severe AEs associated with other PI3K inhibitors, especially hepatic enzytopathy, opportunistic infections, and colitis.

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 were eligible who had relapsed/refractory FL 25 months after last treatment cycle, or within 3 months of last treatment, or with ≤12 months disease progression after last treatment. The primary endpoint is overall response rate (ORR) as assessed by an independent review committee (IRC) per revised International Working Group (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.

Results: 20 pts with iNHL were treated on study. Of these, 83 pts with FL received duvelisib with a median duration of exposure of 6 mo. (range: 0.4 - 24). Median age was 64 years; 68% were male. Most FL pts had an ECOG performance status score at baseline of 0 (51%), followed by 1 (42%) and 2 (7%). Most FL pts (65%) had a FLIPI score at baseline ≥3, and most had either Stage 3 (46%) or Stage 4 (39%) disease. Median time from last anticancer therapy to first dose of duvelisib was 3.2 months. FL pts received a median of 3 prior anticancer regimens (range: 1 - 10); 65% of pts received ≥3 prior regimens, 17% 2 prior regimens. The median time to progression (TTP) was 1.9 mo. (range: 0.4 - 11.7). 80% of FL pts experienced a reduction in nodal target lesions after treatment with duvelisib. Among the 34 FL pts with a response to the median DoR was 9.2 months. The median PFS for all FL pts was 8.3 months, while the median OS was 11.1 months. Among all pts treated on study (n=129), AEs were mostly Gr 1-2. Most common Gr ≥3 AEs were patient-reported neuropathy (39%), fatigue (24%), anemia (12%), and diarrhea (15%). 17% of pts discontinued duvelisib due to an AE. Opportunistic infections occurred in <5% of pts, none were fatal, and included 1 pt (0.8%) with pneumocystis and 3 pts (2.3%) with CMV infections. Six pts had an AE with a fatal outcome.

Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory population (FL 41% ORR, median DoR 9.2 mo., 80% with reduction in target lesions). DUVElisib was generally well tolerated, with a manageable safety profile with appropriate risk mitigation. Duvelisib monotherapy has a favorable benefit-risk profile in double-refractory iNHL, and may represent an important treatment option. Updated clinical data will be available at the time of presentation.
Background: Between March 2000 and May 2005 a multicenter randomized trial comparing frontline use of CHOP-R vs R-HDS with autograft has been performed on 134 Follicular Lymphoma (FL) patients, selected for age less than 60 yrs. and poor prognostic features according to age-adjusted IPI (2-3) and IIL-score (3 or greater). Results at 4-yr follow-up were previously published (Ladetto M et al, Blood 2008), showing superior disease control with R-HDS without any survival advantage.

Aims: We have recently performed a long term update and the results at a median follow-up of 13 yrs are here presented.

Methods: The long-term outcome has been updated for 119 out of the original 134 randomized patients (56 CHOP-R and 63 R-HDS arms). Main features of the updated patients included: median age 51 yrs. (22-60), M/F ratio 68/51, aaIPI 2-3 90%, high LDH 43%, bulky disease 60%, B-symptoms 46%, BM involvement 86%; no significant differences were observed in clinical presentation between the two arms, as previously reported. Treatment schedule consisted of: i. CHOP-R arm: 6 courses of cyclophosphamide/doxorubicin/vincristine/prednisone followed by 4-weekly rituximab courses; ii. experimental R-HDS arm: rituximab with high-dose sequential chemotherapy followed by autografting. The analysis was intention to treat with event-free survival as the primary endpoint. Minimal residual disease (MRD) was evaluated post treatment in 56 patients with a bcl-2/IgH MBR or mcr translocation confirmed at diagnosis by nested PCR. The trial was registered at www.clinicaltrials.gov, no. NCT00435955. The long-term outcome has been updated in January 2017 by 27 out of 30 participating Centers, on 119 patients (88% of the whole series).

Results: Complete remission (CR) was achieved by 86 (72%) patients, including 32 (57%) with CHOP-R and 54 (85%) with R-HDS (p <.001); Molecular Remission (MR) was achieved in 37 out of 56 (66%) evaluable patients. At a median follow-up of 13 yrs., 74 patients (63%) are alive. Overall, 22 patients died for lymphoma progression (13 CHOP-R, 9 R-HDS), 12 died for secondary malignancy (3 in the CHOP-R, 9 in the R-HDS arms), 11 patients died for other causes, including four early toxic deaths. The overall survival (OS) for the whole series is 63% at 13 yrs, as shown in Figure 1A. No significant differences in the long-term OS were observed between the two arms, with 13-yr survival of 65% and 61% for CHOP-R and R-HDS, respectively (p=0.51). At 13 years, the event free survival is 35%, whereas the disease-free survival (DFS) is of 53%, as shown in Figure 1B. Response to induction therapy had a major impact on the OS, with 13 yr survival of 75% for patients achieving CR vs 33% for those with less than CR (p <.001). Similarly, Molecular Remission (MR) achievement was associated with prolonged OS, with 13 yr survival of 81% for patients in MR on BM cells, and of 47% for those with positive MRD (p=.02) (Figure 1C).

Summary/Conclusions: i. poor risk FL may have a prolonged survival, with 63% of patients alive at 13 yrs.; ii. no survival differences between CHOP-R and R-HDS can be detected even at 13 yrs of follow-up; iii. achieving CR is still crucial for the long-term survival; iv. the MRD analysis has a prognostic impact not only on progression-free but also on OS; v. lymphoma progression remains the major cause of death, while secondary neoplasms represent the second cause of treatment failure; vi. a subgroup of advanced-stage FL may experience a prolonged DFS lasting at least 13 yrs: this raises the issue of the potential curability of FL.
Madrid, Spain, June 22 – 25, 2017

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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Background: This study aimed to determine the benefit of early therapeutic
intervention with the combination of elotuzumab, lenalidomide, and dexamethasone in patients with high-risk smoldering multiple myeloma (SMM).
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 this 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
were administered weekly elotuzumab (10 mg/kg) on days 1, 8, 15, and 22 for
the first two 28-day cycles while receiving lenalidomide on days 1-21. For cycles
3-8, patients were administered elotuzumab infusions on days 1, 8, and 15.
Dexamethasone (40mg) was given on days 1, 8 and 15 for 40 of the 50 patients
enrolled. After 8 cycles or best response, patients were given the option to
mobilize with either cyclophosphamide or plerixafor and collect stem cells for
future transplant. Patients were then allowed to continue on maintenance therapy where they were administered elotuzumab (20 mg/kg) on day 1, in combination with lenalidomide days 1-21 of a 28 day cycle. Bone marrow samples of
33 patients were obtained before starting therapy for baseline assessment and
whole exome sequencing (WES) of plasma cells.
Results: In total, 50 patients were enrolled on this study from January 2015
to date, with the participation of eight sites. The median age of patients
enrolled was 62 years (range 29 to 79) with 18 males (36%) and 32 females
(64%). Interphase fluorescence in situ hybridization (iFISH) detected high
risk cytogenetics in 20 patients. The median number of cycles completed is
12 (range 1 to 24). Therapy related grade 3 toxicities included hypophosphatemia (30%), neutropenia (14%), infection (12%), anemia (2%), pulmonary
embolism (2%), rash (4%), and diarrhea (2%). Therapy related grade 4 toxicities included thrombocytopenia (2%), neutropenia (2%) and one instance
of cholecystitis (2%). Stem cell collection was successful in all patients collected to date. Of the 31 evaluable patients that completed the first 8 cycles
of therapy, the overall response rate was 84%, including 2 complete responses (7%), 11 very good partial responses (36%) and 13 partial responses
(42%), and a clinical benefit rate of 100%. None of the patients showed progression to overt MM to date. WES was performed on 25 samples at the time
of initiation of therapy. Recurrent mutations in the MAPK pathway (KRAS,
NRAS) and tumor suppressor gene, TP53, were detected in 32% of the cases
(16% each), while mutations in NF-KB and plasma cell differentiation pathways were present in 10% of patients. CCND1 gene mutation was seen in 1
patient and was associated with t(11:14) as reported in the previous WES
studies of MM. CNAs were called based on WES: 1q amplification, 13q, 17p,
and 1p deletions were identified in 28, 20, 16, and 12% of cases, respectively.
Interestingly, in 6 patients, high-risk CNAs were not reported in iFISH but
were detected by WES. Finally, we assessed the correlation between neoantigen load and clinical response.
Summary/Conclusions: The combination of elotuzumab, lenalidomide, and
dexamethasone is well tolerated and demonstrates a high response rates with
no progression to overt MM to date. Correlation with genomic studies can help
define patients who benefit the most from this early therapeutic intervention.

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Background: Addition of a proteasome inhibitor to a doublet backbone therapy
has been shown to improve efficacy in newly diagnosed multiple myeloma
2017). Data from two phase 1/2 studies indicate that the combination of ixazomib plus lenalidomide-dexamethasone (IRd) is feasible and active in patients
with NDMM, with weekly and twice-weekly ixazomib dosing having been investigated (Kumar et al, Lancet Oncol 2014; Richardson et al, Blood 2013).
Aims: This phase 1/2 study (NCT01383928) evaluated twice-weekly ixazomib
plus Rd as induction therapy, followed by maintenance therapy with singleagent ixazomib. We report long-term efficacy and safety data in patients who
did not withdraw from the study in order to receive SCT.
Methods: Patients with NDMM (SCT-eligible or SCT-ineligible) received twiceweekly oral ixazomib (3.0 or 3.7 mg on days 1, 4, 8, and 11) plus lenalidomide
(25 mg on days 1–14) and dexamethasone (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
received therapy until disease progression or toxicity. Those who proceeded 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
early 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
response-evaluable population was 95%, the complete plus very good partial
response (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
treatment, the ORR (≥PR) was 94%, the CR+VGPR rate was 89%, and the CR
rate was 44%. Two (11%) patients improved their responses during maintenance (1 VGPR to stringent CR, and 1 VGPR to near-CR). Five (28%) patients
who received maintenance therapy had an onset of a grade ≥3 treatment-related AE in cycle 17 or beyond. Rash (aggregate term) was infrequent with single-agent ixazomib during maintenance (1 patient, 6%).
Table 1.

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TWICE-WEEKLY IXAZOMIB PLUS LENALIDOMIDE-DEXAMETHASONE IN
PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA:
LONG-TERM FOLLOW-UP DATA FOR PATIENTS WHO DID NOT
UNDERGO STEM CELL TRANSPLANTATION
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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.

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LENALIDOMIDE INDUCTION AND MAINTENANCE THERAPY FOR TRANSPLANT ELIGIBLE MYELOMA PATIENTS: RESULTS OF THE MYELOMA XI STUDY
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Background: Immunomodulatory agents are effective therapies for multiple myeloma (MM) acting via the modulation of cereblon. Lenalidomide (Len) has fewer side effects than Thalidomide (Thal), whilst retaining the benefits of oral administration, enabling long-term treatment that has been associated with better disease control. Combinations of agents induce deeper, longer remissions by targeting different clonal populations, with triplets outperforming doublets. The optimum immunomodulatory-based induction combinations and maintenance regimens are unknown.

Aims: The UK NCRI Myeloma XI study compared triplet induction regimens of Len vs Thal and evaluated the role of post-ASCT maintenance Len vs observation, enabling a prospective comparison of induction with Len mainteneance.

Methods: Myeloma XI is a multicenter, open-label, parallel group, randomised controlled trial for newly diagnosed MM patients of all ages, with pathways for transplant eligible (TE) and non-eligible patients. For TE patients the induction question compared Len or Thal plus cyclophosphamide and dexamethasone (CTD vs CTD) continued for a minimum of 4 cycles and to maximum response. For patients with a suboptimal response there was a subsequent randomization to a proteasome inhibitor containing triplet or no further therapy, prior to high-dose melphalan and ASCT. A maintenance randomisation at 3 months post ASCT observed Len till disease progression or observation. High risk disease was defined as presence of more than 3 risk factors (CrCl<30mL/min, del(17p), t(4;14), del(17q), 2042 TE patients underwent the induction randomization (CRD 1021, CTD 1021). After a median follow up of 36.3 months, 965 PFS and 415 OS primary endpoint events had occurred. Secondary endpoints include response and toxicity. Results: In TE patients, CRD induction was associated with deeper responses than CTD (76% vs CTD 71%, p=0.005), and CRD maintained the superior induction which persisted post ASCT (≥VGPR CRD 82% vs CTD 77%). This was associated with a significantly improved median PFS. Patients receiving CRD achieved a median PFS of 35.9 months compared to 32.9 for those who received CTD (HR 0.85, 95%CI [0.75, 0.96], p=0.0116). This also translated into an overall survival benefit, 3 year OS: CRD 82.9% vs CTD 77.0% (HR 0.77, 95%CI [0.63, 0.93], p=0.0072).

There were higher rates of PN and constipation with CTD vs haematological toxicity with CRD. Maintenance therapy with Len was associated with a significantly higher median PFS compared to observation (TE HR 0.47, 95%CI [0.38, 0.60]). This finding persisted across all subgroups including patients with high-risk disease, a post-hoc analysis across the TE pathway suggested that CRD induction with Len maintenance was optimum: 60 month PFS CRD-R 50.2%, CTD-R 39.1%, CRD-obs 18.5%, CTD-obs 23.4%.

Summary/Conclusions: In TE patients CRD was associated with deeper responses than CTD and with a PFS and OS benefit. The best outcomes were associated with Len induction plus Len maintenance. Our findings support continuing Len therapy through induction until disease progression.

On behalf of the UK NCRI Haematology CSG.

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COMPARISON OF DENOSUMAB WITH ZOLEDRONIC ACID FOR THE TREATMENT OF BONE DISEASE IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA; AN INTERNATIONAL, RANDOMIZED, DOUBLE BLIND TRIAL
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Background: Multiple myeloma is characterized by osteolytic bone disease, with up to 80% of pts presenting with detectable lesions. Myeloma bone disease is mediated by osteoclast activating factors such as RANKL, increasing the risk of skeletal-related events (SREs) and impacting morbidity and mortality. DMB, a human monoclonal antibody that targets and binds to RANKL, can be administered subcutaneously (SC) to pts regardless of renal function.

Aims: This study evaluates the efficacy and safety of DMB compared with ZA in newly diagnosed myeloma pts.

Methods: Adult pts were randomized 1:1 to DMB 120 mg SC Q4W or ZA 4 mg IV (adjusted) Q4W along with anti-myeloma therapy. Key stratification factors included type of first-line therapy (novel or non-novel) and previous SRE. Pts with renal insufficiency were excluded if baseline creatinine clearance (CrCl)<30mL/min. The primary endpoint was non-inferiority of DMB to ZA with respect to time to first on-study SRE. Secondary endpoints included superiority of DMB for time to first on-study SRE and first-and-subsequent on-study SRE, and overall survival (OS). Progression-free survival (PFS) was an exploratory endpoint. Safety was also assessed.

Results: A total of 1718 pts were randomized, 859 to each arm. Baseline demographics and disease characteristics were balanced, with 66.0% of DMB and 67.2% of ZA pts reporting prior SRE history; CrCl<30mL/min was reported in 26.7% of pts. During the primary blinded treatment period (median follow-up 17.4 months [m]), 43.8% DMB pts and 44.6% ZA pts had a first on-study SRE. The median time to first on-study SRE was similar between DMB (22.83 m) and ZA (23.98 m) pts. DMB was non-inferior to ZA in delaying time to first on-study SRE (HR[95%CI]=0.98[0.85,1.14]). Superiority was not demonstrated for time to first on-study SRE (P=0.82) and time to first-and-subsequent on-study SRE (P=0.84). In this high-risk study population the effect of antiresorptive therapy may only be evident later in the treatment course. A post-hoc, landmark analysis at 15 m for first SRE occurred in 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 (121[14.1%]) than ZA (129[15.0%]). PFS yielded a HR[95%CI]=0.82[0.68,0.99], descriptive P=0.036, with median times of 46.09 m (95%CI:34.3,3.3 NE) for DMB and 35.38 m (95%CI:30.19 NE) for ZA. The most common TEAes≥2%= DMB 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 positively adjudicated ONJ (4.1,2.8) were comparable to known safety profiles. Fewer DMB pts (%) compared with ZA pts had AEs potentially related to renal toxicity (10.0,1.7;P<0.001), most notably in pts with baseline CrCl<30mL/min (12.9,26.4). TEAes led to IP discontinuation in 12.2% of all pts (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.
**PEMBROLIZUMAB PLUS LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE FOR PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: EFFICACY AND BIOMARKER RESULTS FROM THE PHASE 1 KEYNOTE-023 STUDY**


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**Background:** Pembrolizumab (pembro) is a humanized, highly selective, high-affinity IgG4/κ antibody that blocks the interaction between programmed death 1 (PD-1) and its ligands PD-L1 and PD-L2, activating antitumor immunity. Pembro plus lenalidomide (len) and low-dose dexamethasone (dex) may provide synergetic 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 (NCT02036502) 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 orally weekly on each 28-day cycle. Primary end points were safety and determination of the MTD. ORR was assessed by IMWG 2006. Exploratory biomarker analyses included analysis of PD-L1 and PD-L2 on CD38+CD138+ cells in BM aspirate samples obtained at screening, or before the first dose of study drug. Absolute and/or relative numbers of circulating lymphocytes (by flow cytometry [FC]) and gene expression profile (GEP) (by Nanostring) were evaluated in predose; cycle 1, 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 20/40 (50%) (1 sCR, 5 VGPR, 14 PR); 35/40 (88%) patients had a reduction in allele burden over time. The disease control rate (sCR+CR+VGPR+PR+SD) was 39/40 (98%) in the efficacy population and 28/29 (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 samples, 34/35 (97%) patients had a ≥50% reduction in the MPN-SAF TSS. At wk 80, mean percentage change from baseline in JAK2V617F allele burden was -9.7% in the RUX arm (n=65) vs +0.3% in the BAT arm (n=3). AEs observed were consistent with those generally reported with RUX (primarily grade 3/4). Most common grade 3/4 AEs (all G, exposure-adjusted rate per 100 pt-years) were anemia (14.3%), weight increase (10.6%), arthralgia (9.1%), and pruritus (9.1%) in the RUX arm vs pruritus (37.5%), headache (16.9), and thrombocytopenia (15.0%) in the BAT arm. Rate of thromboembolic events (Standardized MedDRA Query, exposure-adjusted) was RUX (1.5%) vs BAT arm (1.9). No pt in the RUX arm had disease progression; 2 pts in the BAT arm. No deaths were reported in the RUX arm vs 3 pts in the BAT arm.

**Summary/Conclusions:** RUX provided durable HCT control, durable CHR, reduction in PBT requirement, improved symptom burden, and was generally well tolerated with >90% of pts still receiving Tx at wk 80. A confirmatory RUX vs BAT phase 3 trial is planned; the results of this study will provide further insight into the role of RUX in RRMM.
Background: Momelotinib (MMB), an investigational oral JAK inhibitor, has been shown in early trials to reduce spleen volume, improve disease associated symptoms and improve red blood cell (RBC) transfusion requirements in patients with myelofibrosis (MF).

Aims: To test the non-inferiority of MMB vs ruxolitinib (RUX) in splenic volume reduction and symptom amelioration, and superiority in transfusion requirement, in JAKi naive patients with primary myelofibrosis, and post-polycythemia vera or post-essential thrombocythemia myelofibrosis.

Methods: Eligibility included primary myelofibrosis or post-polycythemia vera/essential thrombocythemia myelofibrosis; International Prognostic Scoring System (IPSS) high risk, intermediate-2 risk, or intermediate-1 risk associated with symptomatic splenomegaly; palpable spleen ≥5cm; and no Grade ≥2 peripheral neuropathy. Informed consent was obtained. Stratification was by transfusion dependence and baseline TSS (modified MPN-SAF Total Symptom Score) <18 or ≥18. Patients were randomized 2:1 to 24 weeks of open-label MMB 200 mg qd or RUX. Assessments included spleen volume by MRI, and patient reported symptoms using a daily eDiary for TSS. Primary endpoint was spleen response rate (SRR; ≥50% reduction from baseline) before week 24. Secondary endpoints, evaluated sequentially, were rate of TSS response (TSS RR; ≥50% reduction from baseline), RBC transfusion independence and RBC transfusion dependence, and rate of RBC transfusion.

Results: Among 320 patients, 175 of 215 (81%) and 201 of 217 (93%) patients randomized to MMB or BAT, respectively, completed the 24 week double blind phase with MMB were thrombocytopenia (7%) and anemia (13%). Grade ≥3 infections occurred in 7% of MMB and 3% of RUX patients. Treatment emergent peripheral neuropathy occurred in 22 (10%) of MMB (all Grade ≤2) and 10 (5%) of RUX (9 Grade ≤2, 1 Grade 3) patients in the double blind phase, none discontinuing study drug for this problem. Overall, adverse events led to study drug discontinuation in 13% of MMB and 6% of RUX patients in double blind phase. 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

Table 1.

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>MMB</th>
<th>RUX</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen response rate, %</td>
<td>26.5</td>
<td>29.0</td>
<td>0.01*</td>
</tr>
<tr>
<td>TSS response rate, %</td>
<td>28.4</td>
<td>42.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Transfusion independence rate, %</td>
<td>66.5</td>
<td>49.3</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Transfusion dependence rate, %</td>
<td>30.2</td>
<td>40.1</td>
<td>0.19</td>
</tr>
<tr>
<td>Transfusion rate (units/month), median</td>
<td>0.0</td>
<td>0.4</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

3a Test for non-inferiority; b Test for superiority; all values nominally significant.

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

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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; currently or previously treated with ruxolitinib for at least 28 days who either required transfusions or dose reduction to <20 mg BID with at least one of Grade ≥3 thrombocytopenia, anemia, or bleed; palpable spleen ≥5cm; and No Grade ≥2 peripheral neuropathy. Informed consent was obtained. Stratification was by transfusion dependence and baseline TSS (modified MPN-SAF Total Symptom Score >0) ≥18 or <18. 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 for TSS. Primary endpoint was spleen response rate at 24 weeks (SRR>24% ≥35% reduction from baseline). Secondary endpoints, evaluated sequentially, were rates of TSS response (TSS RR> ≥50% reduction from baseline), RBC transfusion independence and RBC transfusion dependence (TD). Results: 73 of 104 (70%) and 40 of 52 (77%) patients receiving MMB or BAT, respectively, completed the 24 week randomized treatment phase. BAT for 80% of 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 (6%) and thrombocytopenia (5%), and in BAT patients, anemia (13%), thrombocytopenia (5%), and abdominal pain (6%). Treatment emergent peripheral neuropathy occurred in 11 (11%) of MMB (1 Grade 3) and in no BAT patients; MMB was discontinued in 3 patients due to neuropathy.

Table 1.

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>MMB</th>
<th>BAT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen response rate, %</td>
<td>6.7</td>
<td>6.8</td>
<td>0.90</td>
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<tr>
<td>TSS RR, %</td>
<td>26.2</td>
<td>5.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Transfusion rate (units/month), median</td>
<td>0.5</td>
<td>0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>TI rate, %</td>
<td>43.3</td>
<td>21.2</td>
<td>0.001*</td>
</tr>
<tr>
<td>TD rate, %</td>
<td>50.0</td>
<td>63.5</td>
<td>0.10</td>
</tr>
</tbody>
</table>

3b-values nominally significant.
Summary/Conclusions: In previously ruxolitinib-treated patients with myelofibrosis, 24 weeks of momelotinib was not superior to best available therapy for splenic response, but significantly better in improving disease related symptoms and transfusion independence. NCT02101268.

S787 MOLECULAR RESPONSE TO HYDROXYUREA AND ROPEGINTERFERON ALFA-2B IN THE PROUD-PV RANDOMIZED PHASE 3 TRIAL

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Background: Interferon alfa (IFNa) has been successfully used to treat myelo proliferative neoplasms (MPN) for many years and several phase 2 studies have demonstrated high rates of hematological and molecular responses assessed by the quantification of mutant JAK2 allele burden (%JAK2V617F). In peripheral blood, BM clonogenic assays suggest that addition, clonal architecture studies showed that the % of JAK2V617F mutant alleles is an important driver of the clonal architecture. Here we report, for the first time, the molecular response of BM progenitors in patients treated with IFNa and hydroxyurea in a phase 3 study.

Methods: Randomized, controlled, multicenter phase 3 trial comparing efficacy, safety and tolerability of hydroxyurea and Ropeginterferon Alfa-2b in PV pts (NCT01949805). The primary endpoint was non-inferiority of AOP2014 versus HU at 12 months (mos) of therapy in terms of complete hematological response (CHR) according to ELN criteria and normal spleen size. An important secondary endpoint the treatment of %JAK2V617F at 12 mos. No complete MR was achieved at 12 mos, but PMR was observed in 45.6% in the subgroup of French pts (54% males, mean age 55 years) CHR at 12 mos was 40% in pts receiving AOP2014 (n=5) and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46% respectively. In the group of pts enrolled in France, we could study BM progenitors clonogenic potential by cultures with or without Erythropoietin (EPO) at baseline and after 12 months of therapy. The presence of colonies without EPO, namely Endogenous Erythroid Colonies (EECs) is a hallmark of PV. After 14 days, erythroid colonies were enumerated and picked for molecular analyses.

Results: A total of 257 pts were randomized in 13 European countries including 13 pts in France. Non-inferiority of AOP2014 versus HU regarding CHR could be demonstrated in the whole study population (43.1 ± 45.6%). In the subgroup of French pts (54% males, mean age 55 years) CHR at 12 mos was 40% in pts receiving AOP2014 (n=5) and 50%, in those receiving HU (n=8). %JAK2V617F at baseline in the AOP2014 and HU arms were 39.4% and 46% respectively. BM progenitors could be studied in 10/13 French pts, 3 treated with AOP2014 and 7 with HU. AOP2014 treatment induced an important decrease of the proportion of EECs (0% at baseline vs 5% at 12 mos). In addition, clonal architecture studies showed that the % of JAK2V617F mutant colonies decreased before and after treatment profoundly decreased in all AOP2014-treated patients (median decrease 25%). Among HU-treated pts, only 1 experienced a decrease in the % of mutated colonies while mean ratio of mutant versus 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 Ropeginterferon 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.

S788 POOLED SURVIVAL ANALYSIS OF MIDOSTAURIN CLINICAL STUDY DATA (D2201+A2213) IN PATIENTS WITH ADVANCED SYSTEMIC MASTOCYTOSIS COMPARED WITH HISTORICAL CONTROLS

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1University Medical Centre Mannheim, Mannheim, Germany, 2University Medical Center Groningen, Groningen, Netherlands, 3University of New Mexico, Albuquerque, 4Brigham and Women’s Hospital, 5Dana-Farber Cancer Institute, Boston, United States, 6University of Paris Descartes, Imagine Institute, Paris, France, 7The Ohio State University Comprehensive Cancer Center, Columbus, 8University of Pennsylvania, Philadelphia, 9Memorial Sloan Kettering Cancer Center, New York, 10Novartis Pharmaceuticals Corporation, East Hanover, United States, 11Novartis Pharma SAS, Rueil-Malmaison, France, 12Novartis Pharma AG, Basel, Switzerland, 13University of Cologne, Cologne, 14Ludwig Maximilians University Munich, Munich, Germany, 15Medical University of Vienna, Vienna, Austria, 16Stanford University School of Medicine, Stanford, United States

Background: Adv/SVM (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 D816 mutations occur in a majority of patients with advSM. Midostaurin is a multitargeted kinase inhibitor that blocks wild-type and D816V-mutated Kit. Two single-arm phase 2 studies (D2201+A2213) evaluated the safety and efficacy of midostaurin in advSM. Overall, 60% and 69% of patients in D2201 and A2213, respectively, achieved the primary endpoint of complete or partial normalization of SM-related organ damage.

Methods: We compared pooled data from these studies with data from a patient registry to determine the effects of midostaurin on overall survival (OS).

Results: Data from the midostaurin studies, in which patients received midostaurin 100 mg twice daily until progression or toxicity, were pooled. Historical control data were obtained from a contemporary patient registry based at University Medical Centre Mannheim, Germany. Although the primary analysis did not include matching for patient subgroup, analyses were performed to assess whether baseline characteristics affected OS and estimated HR. Propensity scoring was used for supportive analyses to match the patients in the registry. Patients were evaluated for OS based on time from diagnosis to death; patients in the pooled analysis with known dates of diagnosis were included in the primary analysis. A sensitivity analysis to compensate for potential bias in patient selection was conducted using the start date of last treatment to death.

Results: The primary analysis of OS in patients with advSM included 89 patients from the midostaurin pooled analysis for whom the date of diagnosis was available (77% of the entire pooled cohort) and all 46 patients from the German registry who had not been treated with midostaurin. SM subtypes among patients from the pooled analysis and registry were similar: 66% of patients in the pooled cohort and 63% in the registry had an AHN (Table 1). Kit D816 mutations were present in 82% of patients in the pooled analysis and 96% in the registry. More patients in the registry (67%) vs the pooled analysis (42%) were aged >65 y. Median follow-up (time from diagnosis to data cutoff for the analyses) was similar for the 2 patient groups: registry, 54.9 (range, 1.9-150.4) mo and midostaurin, 53.6 (range, 31.6-215) mo. Patients in the midostaurin pooled analysis had a clinically relevant improvement in OS versus historical controls (HR=0.62 [95% CI, 0.39-0.98]; P=0.024; Figure 1). Median OS was 42.8 (95% CI, 31.0-52.9) mo in the pooled analysis versus 24.0 (95% CI, 13.0-39.5) mo in the registry. Multivariate Cox regression analysis after adjusting for covariates was consistent with the primary analysis: HR=0.51 (95% CI, 0.30-0.88); P=0.0147. Data using propensity score for matched pairs (n=44) were also consistent (HR=0.381 [95% CI, 0.169-0.960]; P=0.101). Subgroup analyses of OS showed HR in favor of midostaurin for all subgroups analyzed (all subgroups vs >65 y, Kit D816V status, number of prior therapies [≥1 vs <1], and SM subtype) except MCL. Subgroup analysis data should be interpreted with caution due to the small patient numbers in the German registry. Sensitivity analysis of OS from date last treatment received (pooled analysis, n=115; registry, n=42) was consistent with the main analyses (HR from the multivariate analysis=0.38 [95% CI, 0.22-0.65]; P=0.0004).

Table 1.
Background: Approximately 10% of the children with Down syndrome are diagnosed with transient myeloproliferative disorder (TMD) within the first days of life. Previous studies have shown that TMD patients face an around 20% risk of early death and a 20% to 30% risk to develop myeloid leukemia during the first 4 years of life (ML-DS).

Aims: The aim of the AML-BFM TMD Prevention 2007 trial was to analyze the outcome of patients diagnosed with TMD and to evaluate whether the application of a low-dose cytarabine treatment can prevent the progression to ML-DS.

Methods: The AML-BFM TMD Prevention 2007 trial is a multi-center, non-randomized, historically controlled study. Patients with TMD were prospectively enrolled. They received a low-dose cytarabine treatment (1.5 μg/kg i.c. daily for one week) if they met the following criteria: TMD-related symptoms (e.g., hyperleucocytosis, hepatopathy) at diagnosis, MRD-positivity (FACS≥10⁻³ or qPCR≥10⁻⁴) eight weeks after diagnosis. Patients could receive cytarsabin-treatment up to three weeks in case of failure to respond to the cytarsabin-treatment (morphologic detection of blasts between week four and eight after diagnosis and/or MRD-positivity after treatment in week ten after diagnosis).

Results: Here we report a cohort of 108 patients (male: 60, female: 48) diagnosed with TMD. The median age at diagnosis was 4 days. As common in children with Down syndrome, many of the patients presented with comorbidities (cardiac defects: 68%, other malformations: 15%); 36% were delivered preterm. 45 patients received low-dose cytarabine treatment, 57 patients did not receive this treatment. Overall, patients in this trial do not show a significantly better event-free survival (EFS; 72±4% vs 63±4%, p=0.15) and overall survival (OS; 91±3% vs 85±3%, p=0.15) than the historic control group (n=146). The cumulative incidence (CI) of death was lower, (8±3% vs 15±3%) albeit not significantly (p=0.09). The CI of ML-DS was also similar (19±4% vs 22±4%, p=0.88). Patients that presented with TMD-related clinical symptoms (n=43; symptoms: hyperleucocytosis [WBC>100,000], hepatopathy, ascites, hydrops fetalis) had a tendency for a better EFS (59±8% vs 44±6%, p=0.097), OS (80±6% vs 67±7% p=0.10) and CI of death (20±7% vs 33±7%, p=0.10) than patients with those symptoms in the historic control group (n=45). For the progression to ML-DS there is no significant difference between the two groups (21±7% vs 23±7%, p=0.91). For patients that do not show any of the TMD-related symptoms (n=59), no significant differences were observed regarding EFS (81±5% vs 71±5%, p=0.27), OS (98% vs 93±3%, p=0.16) and CI of ML-DS (19±8% vs 22±4%, p=0.95) compared to patients without these symptoms in the historic control (n=101).

Summary/Conclusions: The consequent treatment with low-dose cytarabine of symptomatic patients results in a trend towards reduced CI of death as compared to the historic control. However, progression to ML-DS remained unchanged suggesting that the treatment with low-dose chemotherapy does not seem to prevent the development of subsequent leukemia in TMD-patients. Therefore, a general preventative chemotherapeutic treatment of children diagnosed with TMD cannot be recommended. However, children with TMD-related symptoms should receive low-dose cytarabine to reduce disease-related mortality.

S789

LOW-DOSE CYTARABINE TREATMENT IN CHILDREN WITH DOWN SYNDROME AND TRANSIENT MYELOPROLIFERATIVE DISORDER TO PREVENT ML-DS: AML-BFM TMD PREVENTION 2007 STUDY

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Summary/Conclusions: Midostaurin was associated with a 38% lower risk of death vs historical controls. Benefit was generally consistent across key subgroups.

Figure 1.
Background: AML is a heterogeneous disease based on genetic characteristics with impact on prognosis. So, it becomes necessary to treat patients according to risk-adapted therapies.

Aims: To analyze the results of intensive induction and post-remission treatment in 868 patients with the novo AML enrolled into the CETLAM-03 trial between 2003 and 2012 with a prolonged follow-up (results reported at 10 years).

Methods: Patients on day 1 or 2 induction chemotherapy courses of IDICE-G (idarubicin, intermediate cytarabine (IDC), VP-16 and priming with G-CSF) followed by mitoxantrone and IDC as consolidation therapy. Further treatment was assigned according to the CETLAM risk groups as follows: Favorable risk (FR) defined as favorable cytogenetics according to MRC; autologous stem cell transplantation (ASCT) if leukocyte index (LI) is high, defined as patients in CR after a single induction course, <50x10^9/l white blood cells at diagnosis, normal karyotype and absence of FLT3 internal tandem duplication (FLT3-ITD). Next generation sequencing was performed on 42 genes commonly mutated in AML and MDS in 250 patients treated on the RAVVA trial and correlated with OS and EFS. The presence of ASXL1 (p=0.035) and ETV6 (p=0.033) mutations was found to be associated with improved OS. The presence of ASXL1 and ETV6 was associated with a markedly decreased overall survival is consistent with the hypothesis that induction of cell cycle regulator CDKN2A was associated with a notable reduced overall survival risk stratification but also provides insights into the mechanism of action of AZA.

Summary/Conclusions: In this, the largest such study reported, we demonstrated that mutations in CDKN2A, IDH1 and TP53 are associated with worse OS and EFS and that patients with AML not only can inform patient risk stratification but also provides insights into the mechanism of action of AZA.

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 most patients with FLT3-ITD 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 post FLT3-ITD. Sorafenib(SFB) is a TKI with activity against RAF, VEGF and FLT3-ITD and its use as maintenance therapy after allogeneic SCT has been shown as a promising approach to decrease relapse. Several studies report that SFB maintenance post SCT provides durable complete responses; however, there are also descriptions of sorafenib post SCT triggering acute GVHD, cytopenias, rash and diarrhea.

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 leukemic clone. Although few data are available, the SFB regimen (323 mg/m^2 daily) is a TKI with activity against RAF, VEGF and FLT3-ITD and its use as maintenance therapy after allogeneic SCT has been shown as a promising approach to decrease relapse. Several studies report that SFB maintenance post SCT provides durable complete responses; however, there are also descriptions of sorafenib post SCT triggering acute GVHD, cytopenias, rash and diarrhea.

Background: Azacitidine (AZA) represents an important therapeutic advance in patients with acute myeloid leukemia (AML) and high risk myelodysplasia (MDS) ineligible for intensive chemotherapy. However disease progression after less than 50% CR with maintenance therapy is a common occurrence regardless of strategy. AZA has been postulated to represent a reservoir of resistant disease but the impact of AZA based therapy on LSC numbers has not been studied. An additional factor limiting its clinical utility is the identification of patients likely to achieve a significant clinical benefit and molecular predictors of outcome would improve the rationalisation of this important new agent.

Aims: We wished to study the impact of AZA based therapy on LSC numbers as well as identify molecular predictors of outcome in patients treated on the recently completed UK Trials Acceleration Programme RAVVA randomised Phase II trial which compared AZA monotherapy with AZA/VOR combination therapy.

Methods: The RAVVA trial randomized 259 adults with AML (n=217) and MDS (n=42) to receive AZA monotherapy (AZA (75 mg/m^2) x 7 days every 28 days) or AZA combined with VOR (300mg/m^2) every 28 days for a minimum of 6 cycles. Next generation sequencing was performed on 42 genes commonly mutated in MDS in 250 patients treated on the RAVVA trial and correlated with response. Separately serial immunophenotypic quantitation of leukemic stem/progenitor cells (LSC) was performed in 44 patients.
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 (CRp) 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. CRp: 0.3; 95% CI (0.1-1.3) p=0.1. Overall survival at 24 months was also higher in SFB cases as 100% compared with 60% in control group p=0.035. Only 2 patients relapsed post SCT on SFB maintenance, one with new TP53 mutation at relapse, and other received only <30 days of SFB. However, more than half the patients had disease progression within the control period. The most commonly administered dose was 400 mg daily (5 patients) for 28 days cycle; only 2 patients tolerated higher doses and 6 patients received SFB as 300mg daily or less. There were delays in subsequent cycles in 10 of 12 patients, and the most common reasons for delays included cytopenias, liver function test abnormalities, and fatigue.

Figure 1.
Summary/Conclusions: Sorafenib maintenance is safe and can produce long term durable remissions after allogeneic stem cell transplant in a high risk population with FLT3-ITD mutated AML.

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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 and 4) or single dose (D1) with 7+3 induction therapy (cytarabine 100 mg/m² and daunorubicin 60 mg/m²). Methods: AML patients must be eligible for induction therapy. Response assessments occur on D15 and 28. Second induction and post-remission therapies were per investigator choice and did not include additional 33A. MRD was assessed centrally by bone marrow exam by a multiparametric flow at D15 and D28. Results: Split-dose cohort: 42 patients (median age 45.5 yrs [range, 18-65]) were treated with 33A on D1 and 4 (10+10 [n=4] or 20+10 [n=38] mcg/kg) with 7+3. Most patients had intermediate (50%) or adverse (36%) cytogenetic risk. 19% had secondary AML. 2 patients had hematologic DLTs (lack of recovery of platelets [25K] and/or ANC [500] by D42) and 20+10 mcg/kg was determined to be MTD. The median time to count recovery from D1 of therapy in patients who achieved CR/CRi was 4.9 wks for ANC (≥1K) and 5.1 wks for platelets (≥100K). No non-hematologic TEAEs ≥G3 were reported in >10% of patients; non-hematologic TEAEs of any grade occurring in ≥25% of patients were nau-sea (62%), diarrhea, and constipation (38% each). Of the 42 efficacy evaluable (EE) patients, best responses included 25 CR (60%), 7 CRi (17%), and 5 morphologic leukemia-free state (mLFS; 12%) with a CR+CRi (CRc) rate of 76%; 23 of 25 (94%) responses were achieved in the 1st cycle. Of the patients with blast clearance (CR+CRi+mLFS), 73% (27/37) achieved MRD negative status. Single-dose cohort: To date, 25 patients (median age 58 yrs [range, 38-65]) were treated with 33A on D1 only (30 [n=14] or 40 [n=11] mcg/kg) with 7+3. Patients had intermediate (48%) or adverse (36%) cytogenetic risk. 16% had secondary AML. The median time to count recovery from D1 of therapy was 4.1 wks for ANC (≥1K) and 5.9 wks for platelets (≥100K) in patients who achieved CR/CRi. Four patients had hematologic DLTs, 1 at 30 and 3 at 40 mcg/kg. Non-hematologic TEAEs were consistent with those seen in the D1 and 4 schedule. Of the 24 EE patients, best responses included 12 CR (50%), 6 CRi (25%), and 3 mLFS (13%) with a CRc rate of 75%, achieved in 1st cycle. Of the evaluable patients with blast clearance, 89% (17/19) achieved MRD negative status. The CRc rate at the 40 mcg/kg dose level was 91% (10/11); all 11 patients had blast clearance and 90% (9/10) of evaluable patients achieved MRD negative status. Across schedules (N=67), the CRc 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.
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21-COLOR FLOW CYTOMETRY REVEALS IMMUNOPHENOTYPES ASSOCIATED WITH RESPONSE IN ACUTE GRAFT-VERSUS-HOST DISEASE PATIENTS TREATED WITH THE JANUS KINASE INHIBITOR INCB039110

K. Staser1,*, J. Choi1, J. Khoury1, M. Jagasia2, H. Ali3, G. Schiller4, K. Staser1,*, J. Choi 1, J. Khoury 2, M. Jagasia 3, H. Ali 4, G. Schiller 5, INCB039110 - HOST DISEASE VERSUS INDEPENDENT RISK FACTOR FOR DEVELOPMENT OF INTESTINAL GUT COLONIZATION BY MULTI-DRUG RESISTANT BACTERIA IS AN IMPACT OF HLA DISPARITY ON OUTCOME IN HLA-HAPLOIDENTICAL

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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 VRE, 1% by MRSA, 43% by extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, 27% by carbapenem-resistant Enterobacteriaceae (CRE), 9% by MDR-GNB gut colonization (hazard ratio 2.26; 95% CI, 1.05-4.83, P=0.03). Moreover, MDR-GNB colonized patients had significantly more gas-

Background: Although ~50% aGVHD patients respond to steroids, no consen-

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IMPACT OF HLA DISPARITY ON OUTCOME IN HLA-HAPLOIDENTICAL BONE MARROW TRANSPLANTATION FOLLOWED BY HIGH DOSE POST-TRANSPLANT CYTOTOXIC IMMUNOSUPPRESSION

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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. Sur-

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Aims: Since 2010, patients who lacked a HLA-identical donor have been transplanted from a haploidentical donor in our two Italian institutions. In this large series of patients we aim to verify the real degree of antigen disparity between donor and recipient and whether it impacts on transplantation outcomes.

Methods: All haploidentical transplants performed in two Italian institutions from August 2010 to July 2016 (n = 318) were included. All patients received a myeloablative regimen (MA) followed by unmanipulated bone marrow and high dose post-transplant cyclophosphamide (PT-CY), combined with cyclosporine and mycophenolate. Donors and recipients were typed until 2015, using DNA method (SSO and SBT) for HLA A, B, C, DRB1, DQ and DP at a high resolution level, as defined by EFI standards and by NGS at allelic level in 2016 for the same loci. When applicable (72.3% of patients) members of the immediate family were typed to definitively establish HLA genotype and haplotype identity. Differences in HLA matching were calculated by HVG direction. We evaluated overall survival (OS) and non-relapse mortality (NRM) according to the amount of overall mismatches; also, we analyzed cumulative incidence of grade II – IV aGVHD, moderate-severe chronic GvHD and relapse according to the degree of HLA mismatches in the GvH direction and grade I – III of GvHD in the HvG direction. For analysis purpose, the whole patient population was divided into 2 groups: 0–1 antigen mismatches versus 3–4 antigen mismatches. The same distinction was maintained when analyzing only GVH or HVG directed mismatches. Acute GVHD was calculated at day 100, the other parameters were calculated at a cutoff from 2 year (second year of follow up). OS was estimated using the Kaplan-Meier approach while cumulative incidence was calculated for aGvHD, cGvHD, relapse and NRM.

Results: Median age of patients was 48 years (17-74). Diagnoses included acute myeloid leukemia (130), acute lymphoblastic leukemia (64), lymphoid and myeloid neoplasms (43), myelodysplastic syndrome (33). 144 patients (45%) were transplanted in advanced phase of disease. With a median follow up of 562 days (range 6-2241 days), 2-year OS was 55.7%. Concerning the proportion of “true” haploidentical D/R pairs, 231 out of 318 (72%) couples showed 4 mismatched loci at HLA A, B, C and HLA DRB1 loci. Neither OS nor NRM showed significant correlation with the degree of overall mismatches at 2 years (0-2 mm: 54.2% vs 3-4 mm:58.8%, p=0.58 and 0-2 mm:18.2% vs 3-4 mm:19.1%, p=0.93, respectively). Considering only GVH directed mismatches, no difference was highlighted between low or high HLA mismatch burden in cumulative incidence of aGVHD (12.6% vs 23.9%, p=0.13), cGVHD at 1 year (12.2% vs 14.8%, p=0.84) and relapse (33.3% vs 24%, p=0.26). In this series graft rejection rate was 6.6%; no correlation was observed with the amount of HLA mismatch in the HvG direction.

Summary/Conclusions: In this series, about one third of haploidentical donor/recipient pairs differ for less than 4/8 HLA antigens. Furthermore, in the setting of a MA conditioning with PT-CY the real degree of HLA mismatch observed had no impact on OS, NRM, CI of Relapse and acute and chronic GvHD.

S797 CYCLOPHOSPHAMIDE VERSUS ETOPOSIDE IN COMBINATION WITH TOTAL BODY IRRADIATION AS CONDITIONING FOR ADULTS WITH PH-ALLENT UNDERGOING ALLO-HCT. A STUDY FROM THE ACUTE LEUKEMIA WORKING PARTY OF THE EBMT


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Background: High-risk acute myeloid leukemia (AML) is mainly defined by the presence of determined poor-risk cytogenetic abnormalities and is a standard indication for allogeneic stem cell transplantation (SCT). Nevertheless, high-risk AML is a very heterogeneous group including several subgroups 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 outcome but again the real 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 1p abnormalities (ab(-1p)).

Patients: 144 patients (45%) were included and the subsets of patients with 5q- reported to the EBMT registry as having their first SCT between 2000 and 2015.

Results: Five hundred and one pts, 21% of them with secondary AML, have been included. Median age at SCT was 55 year-old (range, 18-75) and median follow-up was 21 months (range, 2-173). At time of SCT, 338 pts (67%) were in first remission (CR1), 21 pts (5%) were in subsequent remission and 42 (28%) had active disease. Two hundred seventy-seven pts (55%) were transplanted from an unrelated donor (UD) and 224 from a sibling donor. A myeloablative conditioning (MAC) was administered in 45% of the pts and a reduced-intensity conditioning (RIC) in 55% of them. The 2-year probabilities of overall survival (OS) and treatment-related mortality (TRM) were 27% and 20%, respectively. The 2-year probability of treatment-related mortality (TRM) was 20%. The cumulative incidence of grade II-IV graft-versus-host disease (GVHD) was 29% and the 2-year cumulative incidence of chronic GVHD was 32%.
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 abn(17p) (N=90). Group 3 (MK) included 169 pts with -5/5q- and MK but no abn(17p). Finally, group 4 (17p) included pts with -5/5q- and abn(17p) (N=193). The 4 groups were quite similar in terms of characteristic. The 2-year probability of LFS was 39% for group 1, 25% for group 2, 20% for group 3 and only 13% for group 4 (p=0.001). NRM was similar across the groups. In multivariate analysis, factors associated with worse OS and LFS were active disease, age, MK and abn(17p). The corresponding 2-year probability of GvHD and relapse-free survival was 27% for group 1, 17% for group 2, 14% for group 3 and 7% for group 4 (Figure 1).

Biomarkers in ALL

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IDENTIFICATIONS OF NOVEL RECURRENT PU.1 FUSIONS WITH HIGHLY AGGRESSIVE PHENOTYPE IN PEDIATRIC T CELL ACUTE LYMPHOBластIC LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) accounts for 10% to 15% of newly diagnosed cases of childhood acute lymphoblastic leukemia (ALL), arising from the malignant transformation of hematopoietic progenitors primed toward T cell development, as result of a multistep oncogenic process. However, since the prognostic significance of these genetic alterations in pediatric T-ALL is not clear, genetic basis which contributes aggressive phenotype or progression of pediatric T-ALL is still to be elucidated.

Alma; To discover driver genetic events, which involved in the aggressive phenotype of pediatric T-ALL and to identify its novel prognostic markers, we performed integrated genetic analysis in a large cohort of T-ALL case.

Methods: Our cohorts included samples from Tokyo Children's Cancer Study Group (TCCSG) and Japan Association of Childhood Leukemia Study (JACLS). Whole transcriptome sequencing (WTS) was performed in 123 cases. Whole transcriptome sequencing (WTS) was performed in 123 cases. Results: Representative recurrent fusion genes were as follows, SIL-TAL1 (n=25), MLL-ENL (n=5), PICALM-MLLT10 (n=5), and NUP214-ABL1 (n=2). Intriguingly, novel recurrent in-frame PU.1 fusions (STM11-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 TLX1, and TLX3 (TLX-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 ETV6. Of note, significant poor outcome was confirmed by multivariate analysis in cases with PU.1 high cluster (p=0.048). Consistently, we defined PU.1 overexpression cases as outliers of PU.1 expression, which resulting in extremely poor prognosis (3-year OS 21%, log-rank p=6.9 × 10^-7).

Summary/Conclusions: PU.1 fusions expressing cells expanded and they remained at an immature stage, implicating a potential leukemogenic activity of these fusions. Not only the cases with PU.1 fusions, but also the cases with
high PU.1 expression without fusions showed extremely poor prognosis, suggest-
ging the prognostic value of aberrant PU.1 expression in pre-B-ALL. Although it remains unclear, why cases with PU.1 fusions/PU.1 expression have a poor prognosis, our results indicate that these cases are genetically distinct subgroup from other pediatric T-ALL.

**S800**

**PROGNOSTIC IMPACT OF ADDITIONAL MOLECULAR LESIONS IN PH+ ACUTE LYMPHBLASTIC LEUKEMIA**

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Background: The outcome of Ph+ acute lymphoblastic leukemia (Ph+ ALL) is drastically improved since the introduction of tyrosine kinase inhibitors (TKI). At present however, well-defined prognostic markers, beyond the moni-
toring of minimal residual disease (MRD) during follow-up and to a lesser extent IKZF1 deletions, are lacking.

Aims: To identify genomic lesions of prognostic value, we evaluated copy num-
ber aberrations (CNA) by SNP arrays, confirmed them by multiplex ligation-
dependent probe amplification (MLPA) and we set up a droplet digital PCR (ddPCR) assays for additional lesions. Furthermore, we correlated the lesions identified with MRD monitoring, outcome and biological features, such as type of fusion protein (p190 or p210). Finally, in a subset of patients gene expression profiling (GEP) was carried out.

Methods: Genomic DNA of 116 newly diagnosed adult Ph+ ALL patients enrolled in 4 consecutive GIMEMA-trials, namely 2010B, 0904, 1205 and 1509, was evaluated. All the trials were based on an induction with steroids and TKI, the first 2 with imatinib and the remaining with dasatinib. For CNA, the Cytoscan HD Arrays (Affymetrix, Santa Clara, CA) were used. The lesions were confirmed by MLPA on all samples using the Salsa MLPA P335-43 ALL-
IKZF1 kit (MRC-Holland, Amsterdam, The Netherlands), ddPCR was used to validate lesions targeting MEF2C. In 42 cases, GEP experiments were per-
fomed using the HGU133 Plus 2.0 gene chips (Affymetrix, Santa Clara, CA).

Results: We found a similar load and type of lesions across the 4 trials, one of which included elderly. The majority of lesions targeted IKZF1 (84%), PAX5 (36%) and CDKN2A/B (32%). In our cohort, IKZF1 deletions alone did not affect com-
plete remission (CR), however, combined (CR) achieving disease-free survival (DFS) was less for patients harboring CDKN2A/B and PAX5 deletions had a significant inferior outcome (p=0.004, p=0.003 respectively). In line with this, a DFS was observed for the so-called “IKZF1 plus” cases, i.e. concomitant deletions of IKZF1 and CDKN2A/B and/or PAX5 (46% vs 24% at 36 months, p=0.005). MLPA con-
firmed the incidence of these deletions and allowed the study of IKZF1 isoforms. Among IKZF1 deleted cases, patients carrying the Δ7 isoform (25%) had a worse DFS (p=0.02) than patients harboring other IKZF1 isoforms. Importantly, SNP arrays highlighted novel genomic lesions targeting MEF2C in 13% of cases, which were associated to the achievement of a CR (p=0.05) and had a significa-
nt impact on DFS (62% vs 32% at 36 months, p=0.02). The association with CR was not affected by the trial (p=0.76) or the TKI used (p=0.57). This result was confirmed by ddPCR. Unsupervised hierarchical clustering of GEP experi-
ments identified 3 subgroups: the first comprised mainly patients who reached a 
CR, the second one the patients who had IKZF1 alone, and the last one comprised patients with Δ7 deletions. Analysis showed an overexpression of genes involved in cell communication and protein modification process in PAX5 deleted cases, suggesting that these genes could be contributing factors in BCR/ABL1-driven leukemia.

Summary/Conclusions: In adult Ph+ ALL, IKZF1 deletions have a prognostic impact similar to other lesions. Among IKZF1 deletions, only the Δ7 deletion has a deleterious effect. MEF2C lesions carry prognostic implica-
tions, being significantly associated with a better prognosis. This study paves the way to design a prognostic model for adult Ph+ ALL that includes these findings and more conventional features, in order to better stratify patients at diagnosis and to further optimize treatment.

**S801**

**MULTICENTER VALIDATION OF STANDARDIZED NGS ASSAYS FOR RERARRANGED IG / TR MARKER DETECTION IN ACUTE LYMPHBLASTIC LEUKEMIA – A REPORT OF THE EUROCLONALITY-NGS CONSORTIUM**


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Background: Amplicon-based next generation sequencing (NGS) of immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements can be used to identify suitable markers for subsequent quantification of minimal resid-
ual disease (MRD) in acute lymphoblastic leukemia (ALL). Within the Euro-
clonality-NGS Consortium we established and validated a standardized quality controlled amplicon-based NGS application to detect clonally rearranged IGH, IGK, TRB, TRG and TRD genes in lymphoid disorders.

Aim: To test EuroClonality TR IG / TR NGS panel within an international multi-laboratory pilot for their suitability to identify clonal markers in ALL in diagnosis, and 2) to compare these NGS results with conventional Sanger sequenc-
ing (SS) of Genescan or Heteroduplex peaks/bands local multiplex PCRs

Methods: Within the EuroClonality-NGS Consortium, V, D, and J gene-spe-
cific primers targeting IGH, IGK, TRB, TRG, TRD sequences were tested with 6 different international laboratories, all members of the EU-CLONALITY network. They were spiked into all samples for calculation of clonotype copy numbers. A standard patient marker quality control (QC) DNA was used to monitor inter- and intra-
lab NGS reproducibility. Results of NGS based marker identification were com-
pared to results of routine SS results.

Results: Fifty ALLs (29 BCP-ALL and 21 T-ALL) were analysed. A total of 10 libraries were deep sequenced, leading to 47 high quality reads (n=9.2 Mib). Pre-processing, identification and immunogenetic annotation of target sequences, quality control and copy number calculations were per-
formed with ARRES/Intergate, using IMGT germine sequences – further analyses and verifications were performed with Vidi/ and IMGT/V-QUEST. Over 70% of all ALL NGS primer sets, and 228/339 (68%) clonal sequences were concordant between both methods. NGS iden-
tified additional clonal rearrangements in 86 (26%) libraries, whereas 23 (7%) clonal markers were only detected by SS. NGS primers covered possible IG/IGK/IGL rearrangements, and 116 (34%) TCR gene rearrangements, and enabled sequencing of bi-allelic rearrangements and weak PCR products. Currently discrepant cases are analysed in more detail using allele-specific PCR assays. Assay performance was analysed by standardized eval-
uation of QC samples and showed high intra- and inter-lab consistency with-
out practically significant differences between the labs.

Summary/Conclusions: The IG / TR NGS panel, as established by the Euro-
clonality-NGS Consortium, allows for quality controlled high-throughput detec-
tion of clonal IG / TR rearrangements in ALL. Compared to low throughput meth-
ods more clonal MRD markers are identified, sensitivity is increased, process-
time is reduced and labour intensive conventional methods to resolve mixed 
sequences in case of bi-allelic rearrangements or background are avoided.

**S802**

**POST-INDUCTION MRD PREDICTS HIGH RELAPSE RISK FOLLOWING REDUCED INTENSITY CONDITIONED ALLOGENIC STEM CELL TRANSPLANTATION: A RETROSPECTIVE STUDY OF ADULT ALL (UKALL14.ISRCTN 66543117)**

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Background: High-risk adult acute lymphoblastic leukaemia (ALL) patients are presented with high relapse risk following post-remission con-
ditioned allogeneic stem cell transplantation (micro- or mini-CIT). In a recent retrospective study of 63 patients, an association was identified between the risk of relapse and the occurrence of minimal residual disease (MRD) at post-transplant day 42 (PTD). We demon-
strated that patients with high-risk MRD status at this time point were at high risk of relapse. In this study, high-risk MRD status was defined as MRD

Results: In a recent retrospective study of 63 patients, we identified the occur-
dence of high-risk MRD status at the timepoint of PTD42 as a statistically sig-
ificant independent predictor of high-risk relapse.

Summary/Conclusions: High-risk MRD status at PTD42 is an independent pre-
dictor of high-risk relapse following reduced intensity conditioned allogeneic stem cell transplantation.
Background: Reduced intensity conditioned allogeneic haematopoietic stem cell transplant (RICalloHCT) enables HCT to be performed in older patients. The UK NCRI UKALL14 study of adult acute lymphoblastic leukaemia (ALL) considers patients ≥41 years “high risk” and recommends a RICalloHCT where there are high quality donors. Other “high risk” factors are high WBC at presentation, t(9;22), t(4,11), hypodiploid/near triploid, complex karyotype and positive minimal residual disease (MRD) after completing induction therapy. The presence of MRD at this time-point predicts poor outcome after conventional chemotherapy. There is evidence that myeloablative alloHCT can overcome this, 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 ≥5x10^-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>Patient characteristics</th>
<th>n=115</th>
<th>Disease characteristics</th>
<th>n=115</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at registration median(range)</td>
<td>45 (20-65)</td>
<td>B-ALL</td>
<td>100 (17)</td>
</tr>
<tr>
<td>Preexisting WBC median(range)</td>
<td>8.6 (0.073)</td>
<td>T-ALL</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Risk N (%)</td>
<td>61 (53)</td>
<td>AML</td>
<td>35 (38)</td>
</tr>
<tr>
<td>Male</td>
<td>54 (47)</td>
<td>Female</td>
<td>56 (52)</td>
</tr>
<tr>
<td>FLT3/ITD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KIT mutation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibling</td>
<td>40 (34)</td>
<td>Complex karyotype</td>
<td>32 (28)</td>
</tr>
<tr>
<td>Medical indication</td>
<td>50 (43)</td>
<td>RICalloHCT catenary risk group N (%)</td>
<td>50 (43)</td>
</tr>
<tr>
<td>Post induction MRD N (%)</td>
<td></td>
<td>Standard</td>
<td>42 (36.3)</td>
</tr>
<tr>
<td>Positive/POQR</td>
<td>71 (60)</td>
<td>Unknown</td>
<td>33 (28.4)</td>
</tr>
<tr>
<td>Positive</td>
<td>38 (33)</td>
<td>Unknown</td>
<td>20 (17.0)</td>
</tr>
</tbody>
</table>

At 2 years post transplant, overall survival (OS) was 63.1% in the 115 patients with evaluable MRD and 62.7% in the 184 patients receiving RICalloHCT; event free survival (EFS) was 55.2% and 55.9% respectively. By contrast, in the 38 of 115 patients with positive MRD after induction, OS and EFS were 40.6% and 28.4% respectively. Twenty eight of the 115 patients relapsed, with a 2 year actuarial relapse risk of 31.5% (22.2-43.5). We assessed the association of the following factors; age, sex, immunophenotype, presenting WBC, BCR/ABL1, other cytogenetics, post-induction MRD and donor type with the risk of relapse. Among this population of high risk patients, post-induction MRD was the only independent prognostic factor for relapse (univariable HR: 3.82 (1.59-9.16), p < 0.001 (see Figure 1) and multivariable HR: 3.82 (1.59–9.16), p = 0.001 (see Figure 1) and multivariable HR: 3.82 (1.59–9.16), p = 0.003). The relapse rate of the MRD+ patients was 57.2% at 2 years post HCT.

Figure 1.

Summary/Conclusions: The 2-year OS of 62.5% in UKALL14 participants over 41 years old after RICalloHCT is greater than would be expected with chemotherapy alone. However, MRD positivity after induction is associated with significantly lower OS, EFS and a higher risk of relapse, which is not abrogated by RICalloHCT.

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**S803**

T-CELL RECEPTOR B REPERTOIRE CHARACTERISTICS IN RELAPSED/REFRACTORY B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA ON BLINATUMOMAB TREATMENT

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1contributed equally, 2Department of internal medicine II, Laboratory for Hematological Diagnostics, University Hospital Schleswig-Holstein, Kiel, Germany, 3Central European Institute of Technology, Brno, Czech Republic

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, an in-depth 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 (114x at time of screening (scr), 74x C1D15, 58x C1D29) of 114 rr Ph-negative BCP-ALL patients (median age: persisters 47; responders 42; p-value=0.81). Patients received Blinatumomab within the phase II trial (MT103-211). Sequencing libraries were prepared using 100ng of DNA via 2-step PCR and sequenced on the Illumina MiSeq (2 x 250bp) with a median coverage of 117,563 reads (range 59,512 – 447,767 reads) per sample. In the first PCR virtually all TRB rearrangements present in the investigated sample were amplified using universal V(D)- and J-regions primers. In the second step, sequencing adaptors and sample-specific barcodes were added. Annotation of V(D)- and J-regions of TRB sequences was performed using ARResT/Interrogate (Bystry, Bioinformatics, 2016). Diversity of TRB repertoire within patient groups and time points was expressed as the Shannon index, using the R-package vegan. Analysis of variance was employed to assess statistically significant differences in diversity between groups and time points.

**Results:** Diversity of TRB repertoire (Figure 1) was significantly higher in responders at time of scr (p=0.02) and at C1D29 (p=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).

Figure 1.
**Summary/Conclusions:** We showed that Blin responders have significantly higher TRB repertoire diversity at scr compared to persisters and that the repertoire expansion during Blin treatment is sharper in responders. Other repertoire characteristics did not differ significantly between groups. Further studies on larger patient cohorts are necessary in order to elucidate whether the response to treatment can be predicted by repertoire diversity at scr.

Amplicon NGS is a useful tool for monitoring of T-cell repertoire. Development, standardization, and validation of TRB primer sets is in progress within EuroClonality-NGS Consortium.

**Research Support:** Amgen.

**Infectious diseases, supportive care**

**S804**

**DISCONTINUING ANTIBACTERIAL THERAPY AFTER APYREXIA AND CLINICAL STABILITY REGARDLESS OF NEUTROPHIL COUNT IN FEBRIL NEUTROPIA IS SAFE AND REDUCES EXPOSURE TO ANTIBIOTICS (HOWLONG RANDOMIZED TRIAL)**

I. Espigado1,*, M. Aguilar-Guisado2, A. Martin-Peña3, C. Gudía3, J. Falantes4, L. Vázquez5, I. Montero6, M.L. Martino6, R. Parody7, J. Gonzalez-Campos8, S. Garzon8, C. Calderon-Cabrera1, P. Barbà9, N. Rodríguez-Torres1, M. Rovira3, J.A. Perez-Simon10, J.M. Cisneros-Herreros1

1Unidad Clínica de Hematología, 2Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva, Instituto de Biomedicina de Sevilla (IBiS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, 3Servicio de Enfermedades Infecciosas, Hospital de Bellvitge, Barcelona, 4Servicio de Hematología, Hospital Clínico de Salamanca, Salamanca, 5Servicio de Hematología, Hospital de Jerez de la Frontera, Jerez, 6Servicio de Hematología, Hospital Universitario Vall’Hebron, 7Servicio de Hematología, Hospital Clinic, Barcelona, Spain

**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.**

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<thead>
<tr>
<th>Variables</th>
<th>EG (n=78)</th>
<th>CG (n=79)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of neutropenia</td>
<td>14 (6.5-24)</td>
<td>11 (8.21)</td>
<td>ns</td>
</tr>
<tr>
<td>Days of fever</td>
<td>(2-9)</td>
<td>(4-28)</td>
<td>ns</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>18 (12.5-215)</td>
<td>18 (9.7-20.2)</td>
<td>0.047</td>
</tr>
<tr>
<td>Per protocol population</td>
<td>EG (n=66)</td>
<td>CG (n=68)</td>
<td></td>
</tr>
<tr>
<td>Days of fever</td>
<td>4 (1-14)</td>
<td>5 (2-9)</td>
<td>ns</td>
</tr>
<tr>
<td>EAT-free days*</td>
<td>19 (14-22)</td>
<td>14.5 (8.7-20)</td>
<td>0.02</td>
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<tr>
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<td>EG (n=36)</td>
<td>CG (n=39)</td>
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<tr>
<td>Days of fever</td>
<td>3 (1-72)</td>
<td>3 (1-57)</td>
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</tr>
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**ITT:** Intention to treat: EAT: empirical antibacterial therapy: EG: experimental group; CG: control group. IQ range: interquartile range.

*EAT-free days: days of follow-up (28) – days of EAT.

**Table 1.**

| Days of neutropenia            | 14 (6.5-24) | 11 (8.21) | ns    |
| Days of fever                  | (2-9)     | (4-28)    | ns    |
| EAT-free days*                 | 18 (12.5-215) | 18 (9.7-20.2) | 0.047 |
| Per protocol population        | EG (n=66) | CG (n=68) |       |
| Days of fever                  | 4 (1-14)  | 5 (2-9)   | ns    |
| EAT-free days*                 | 19 (14-22) | 14.5 (8.7-20) | 0.02  |
| If modified per protocol population | EG (n=36) | CG (n=39) |       |
| Days of fever                  | 3 (1-72)  | 3 (1-57)  | ns    |

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<tr>
<td>Days of fever</td>
<td>3 (1-72)</td>
<td>3 (1-57)</td>
<td>ns</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 LYMPHOCYTIC LEUKEMIA
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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 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 titer (GMTs). Geometric mean concentrations of serotype-specific IgG antibodies elicited by PCV13 as measured by ELISA, were higher than those elicited by PPSV23 in half of the common serotypes, both after one and six months. The proportion of patients with good response (defined as response in 8 of 12 common serotypes according to predefined response criteria) was higher in PCV13 recipients than in PPSV23 recipients after one month (40% vs 22%, p=0.034) as well as after six months (33% vs 17%, p=0.041). Never did PPSV23 trigger a better immune response for any of the serotypes, than PCV13, regardless of analysis. For two of the serotypes, OPA GMTs were lower than the six months than at the one-month follow up. Negative predictive factors for vaccination response were hypogammaglobulinemia and long disease duration. Both vaccines were well tolerated.

Summary/Conclusions: In patients with previously untreated CLL, the efficacy and immune response is superior to PPSV23 for many serotypes common for the two vaccines. PCV13 should be considered as a part in vaccination programs against Streptococcus pneumoniae for these patients and administered as possible during the course of the disease.

S806
INFECTION-RELATED MORTALITY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: AGE, CMV AND PRE-TRANSPLANT LEVELS OF IGA/IGM PREDICT IRM IN A NEW CLINICO-BIOLOGICAL SCORING SYSTEM
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1IRCCS San Raffaele Scientific Institute, 2Vita-Salute San Raffaele University, Milan, Italy

Background: Infection-related mortality (IRM) is a major challenge after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The aim of this study was to develop a scoring system predicting IRM based exclusively on pre-transplant data.

Methods: A total of 589 adult patients receiving allo-HSCT were studied (Jan 2009-Dec 2011). Briefly, 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 <0.350 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 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-years IRM were 5% (95% CI 2.10) and 9% (95% CI 4.16) for low-risk, 11% (95% CI 5.18) and 23% (95% CI 14.33) for intermediate-risk, and 16% (95% CI 16.37) and 41% (95% CI 28.53) for high-risk patients, respectively (P=0.001). In the retrospective validation set, 100-day and 2-yr IRM were 7% (95% CI 3.14) and 14% (95% CI 2.28) for low-risk, 17% (95% CI 10.26) and 23% (95% CI 15.33) for intermediate, and 28% (95% CI 15.42) and 33% (95% CI 19.4) 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, only 100-day IRM was different among the 3 groups, being 59% (95%CI 52.67-62.5) 50% (95%CI 43.59-57.9) and 37% (95%CI 29.48-48) 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=0.03). 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 (2%) to parasites.

Figure 1.

Summary/Conclusions: This new clinic-biological score based on age, CMV serostatus and levels of IGA and IGM, may contribute to the prompt identification of patients at higher risk of fatal infections prior to allo-HSCT, thus promoting post-transplant personalized intensive active surveillance strategies and immune-intervention approaches to improve the overall outcome of transplant. A multicentric Italian study is currently on the way for the external validation of these results.

S807
LETTERMOVIR FOR PREVENTION OF CYTOMEGALOVIRUS INFECTION IN ADULT CMV-SEROPOLITIVE RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION
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1BMT Programme & Myeloid Leukemia Division, Hospital Universitario Puerta de Hierro, Madrid, Spain, 2Division of Hematology, Dana-Farber Cancer Institute, Boston, United States, 3Hematology, Karolinska University Hospital , Stockholm, Sweden, 4Department of Infectious Diseases, University of Bern, Switzerland, 5Hematology, Universitätsklinikum Würzburg, Germany, 6Medicine, Tufts Medical Center, Boston, 7Infectious Disease Division, University of Maryland School of Medicine at the University of Pennsylvania, Philadelphia, United States, 8Internal Medicine, Universitätsklinikum Würzburg, Germany, 9Vaccine and Infectious Diseases Division, Fred Hutchinson Cancer Research Center, Seattle, 10Late Development Statistics, 11Global Clinical Development - Infectious Disease, Merck & Co., Inc., Kenilworth, United States

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<0.001) by Week 24 post-HCT. Figure 1 shows the time to CS-CMV analysis. The most common AEs (LET, placebo) were GVHD (39%, 39%), diarrhea (26%, 25%), and nausea (27%, 23%). More frequent vomiting (19%, 14%), edema (15%, 9%), atrial arrhythmias (10%, 5%), and ALT levels >5xULN (4%, 2%) was noted in LET-treated subjects; no increased myelotoxicity or nephrotoxicity was observed. The Week 24 all-cause mortality was 10% for LET recipients and 15% for placebo recipients.

**Support:** Jazz Pharmaceuticals.
Iron: Deficiency and overload

LACK OF THE FERROPTOSIS INHIBITOR GPX4 IN ERYTHROID CELLS CAUSES A BLOCK IN RETICULOCYTE MATURATION AND A HYPOXIA 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 17 due to tissue-specific ablation 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 large amount of iron to bind and deliver oxygen to all tissues. Being constantly exposed to oxygen, erythroid cells need to continuously fight against oxidative stress by expressing a variety of antioxidant enzymes, including GPX4. Iron availability for erythropoiesis depends on systemic iron levels which are regulated via the hepcidin/ferroportin regulatory system. Hepcidin binding to the iron exporter ferroportin reduces systemic iron export regulating body iron levels. In hypoxic conditions the erythroid hormone ErFe suppresses hepcidin synthesis to provide iron for the elevated erythropoietic demand.

Aims: The aim of this study is to identify how the lack of GPX4 in the hematological compartment affects iron homeostasis.

Methods: Lethally irradiated C57BL/6 female mice were reconstituted with bone marrow cells from Gpx4−/−, Rosa26-CreERT2 or Gpx4−/−,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-consuming diet. Erythrocyte cells have been analysed in FACS. Serum iron levels have been assessed using the SFBC and UIBC iron kits (Biolabo). Gene expression analysis has been performed using SYBR-green qRT-PCR. Circulating Hepcidin has been measured with a specific murine ELISA kit (Intrinic Lifesciences). Tissue iron levels have been measured with a colorimetric assay. All animal experiments were approved by the institutional guidelines in silico, in vitro and in vivo studies. Results: Compared to GPX4−/−,CreERT2 controls, Gpx4−/−,CreERT2 transplanted mice lacking GPX4 in the haematological compartment show a decrease in the number of red blood cells, haemoglobin and haematocrit. Reticulocytosis was detected in bone marrow and spleen specimens, indicating an increase in reticulocytes with a concomitant increase in erythrocyte cell production. Hematocrit 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.

Identification of guanosine 5′-diphosphate as potential iron mobilizer: Preventing the hepcidin-ferroportin interaction and modulation of the interleukin-6/STAT-3 pathway

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Background: Anemia of inflammation (AI) is one of the most common manifestations of iron deficiency in the patients with inflammatory conditions. AI is responsible for hypoferraemia, with consequent iron-restricted erythropoiesis with high level of hepcidin, which stimulate the internalization of ferroportin (FPN) transporter. Therefore, inhibiting hepcidin-mediated FPN degradation can be an important strategy to ameliorate AI.

Aims: To increase iron bioavailability we selected a Novel compound against hepcidin action through natural compound libraries that might provide a new alternative approach to increase iron absorption for prevention of hepcidin-mediated FPN internalization and to ameliorate turpentine-induced anemic state with different insilico, invitro and invivo studies.

Methods: Using 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 (GFPP, FFPN, 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 in vivo studies showed that co-administration of GDP and ferrous sulphate (FeSO4) significantly improved the turpentine-induced anemic state with increase in haemoglobin level (Figure 1B).

Summary/Conclusions: AI is a normocytic anemia, common among patients with chronic infection and inflammatory disorders. We found that GDP molecule have higher contribution to the stability of hepcidin-GDP complex and thus blocks its interaction with FPN. The results support the novel hypothesis that GDP along with iron supplement regimes can overcome the binding of hepcidin from interaction with FPN that would be an effective treatment for AI.

Unraveling the molecular pathogenesis of ineffective erythropoiesis in congenital dyserythropoietic anemia type II (CDAII) by in vitro evaluation of RAP-011 treatment

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Background: Congenital Dyserythropoietic Anemias (CDAs) are subtypes of bone marrow failure syndromes, hallmarking 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 loss of function 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-dependencicas.

Recently, members of TGF-β superfamily have been studied as potential regulators of erythropoiesis especially the growth differentiation factor 11 (GDF11). Through the binding of specific receptors, GDF11 leads to an inhibited late-stage erythropoiesis. Indeed, two GDF11 inhibitors, ACE-011 and ACE-536, have been associated to hematological improvement.

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.
Aims: The main aim of our study is to assess the effects of RAP-011 on different cellular models of CDAII.

Methods: We measured circulating GDF11 levels in CDAII patients and healthy controls (HC) by western blot (WB). To assess the effectiveness of RAP-011 (provided by Celgene Corporation) in vitro, we established two different cellular models of CDAII: (i) K562 cells stably silenced for SEC23B by sh-RNA carried in the pRS vector, and (ii) K562 SE8-SEB-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 levels in secondary culture from 18 CDAII patients compared to HC (p<0.02). Stable silencing of SEC23B in K562 cellsled to the establishment of two different clones, Sh-70 and Sh-74, showing amarkededuction of SEC23Bexpression compared to Sh-CTR (85%-90% and 60-65%, respectively). At 3 and 6 days of K562 erythroid differentiation by hemin, we observed an increase of pSMAD2 in SEC23B-detailed cells compared to non-treated ones; interestingly, a reduction of pSMAD2 in RAP-011+GDF11-treated cells was observed.

Summary/Conclusions: We firstly demonstrated the increased levels of GDF11 in CDAII patients. Thus, we used a combined treatment with hemin+GDF11 in SEC23B-silenced K562 stable clones, in order to reproduce the pathologic phenotype of the disease, and to make K562 cells suitable for RAP-011 treatment, as attested by the increased expression of pSMAD2 in GDF11-treated cells. The reduced pSMAD2 in RAP-011+GDF11-treated cells suggests that RAP-011 treatment leads to repression of ActRIIA/B pathway, which in turn prevents the nuclear levels of GATA1 transcription factor. This action should lead to an increased expression of GATA1-activated genes involved in erythroid development. The evaluation of GATA1 activation is ongoing, as well as the in vitro treatment of K562 stably overexpressing SEC23B-WT,SEC23B-R14W and -E109K.

### S812

**INTRAVENOUS IRON VERSUS ORAL IRON VERSUS NO IRON WITH OR WITHOUT ERYTHROPOIESIS-STEMULATING AGENTS FOR CANCER PATIENTS WITH ANAEMIA: A SYSTEMATIC REVIEW AND NETWORK META-ANALYSIS**

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**Background:** A widely prevalent complication in patients suffering from cancer is the deficiency of haemoglobin-containing red blood cells, referred to as anaemia. While many patients develop anaemia due to an involvement of malignant bone marrow cells, others suffer from so-called chemotherapy/ radiotherapy-induced anaemia. Erythropoiesis-stimulating agents (ESAs) stimulate the production of red blood cells within the bone marrow and have shown to increase Hb levels in anaemic patients. Uncertainties remain regarding the effect of iron supplementation on the fatal consequences of ESA-treatment.

**Aims:** The aims of this systematic review and network meta-analysis are to evaluate the effects of ESAs and iron for the treatment of disease-related as well as therapy-induced anaemia in cancer patients.

**Methods:** Based on an a-priori Cochrane protocol, we developed sensitive search strategies for Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, databases of ongoing trials and conference proceedings (search date 12/2016). We included only randomized controlled trials (RCTs) including anaemic patients of any age with solid and/or haematological malignancy undergoing chemotherapy, radiotherapy or no anti-cancer therapy. We excluded studies including anaemic cancer-patients as a result of surgery or due to haemolysis. Two authors independently assessed studies for eligibility, extracted data and assessed quality of trials. The primary outcome was on-treatment study mortality. Secondary outcomes included number of red blood cell transfusions and thromboembolic events. For binary outcomes, we used risk ratios (RRs) with corresponding 95% confidence intervals (CIs) to evaluate the treatment effects. We performed a random-effects meta-analysis for direct comparisons and a network meta-analysis for indirect comparisons, using the frequentist graph theoretical approach. Treatment hierarchy was obtained giving P-scores on a scale from 0 (worst) to 1 (best).

**Results:** We identified a total number of 105 eligible studies, including 25,722 patients. The network analysis of the primary outcome, on-study mortality, including studies with 8 treatment arms, was performed. When results were pooled, we observed a significant heterogeneity across studies (I² = 92%). The net treatment effect was significant (RR=0.70 (95% CI 0.53-0.92) P-score: 0.87). Further subgroup analyses revealed that ESA effect was more pronounced in studies with low risk of bias.

**Summary/Conclusions:** While our analyses show that ESA use increases mortality and risk for thromboembolic events, there is no evidence that iron administration alters these risks. However, addition of iron to ESA does further decrease the need for RBC-transfusions compared to ESA alone. Further investigation, with regards to iron type and route of administration may yield further distinct results.

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**S813**

**DIFFERENT IRON SOURCES AND ACQUISITION PATHWAYS SHAPE MACROPHAGES TOWARDS OPPOSING FUNCTIONAL PHENOTYPES**

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**Background:** Iron homeostasis and macrophage biology are closely inter-connected. 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.

**Aim:** Increasing evidence suggests that iron availability itself has significant effects on immune effector functions and macrophage polarization. However, it is still unclear how different iron sources and acquisition pathways affect macrophage phenotypes.

**Methods:** To investigate this aspect, we analyzed both in vivo and in vitro, and compared the phenotypic switching of macrophages induced by different iron sources, including heme and iron, as well as hemolytic or intact RBCs.

**Results:** Hemolytic RBCs, free heme and iron-dextran treatment in mice shape macrophage polarization towards an M1-like pro-inflammatory phenotype. Splenic and hepatic macrophages from treated mice show iron deposition and increased expression of iron-related genes (ferroportin, ferritin, HO-1). Moreover, in these cells, the expression of M1 markers such as MCH, CD86 and pro-inflammatory cytokines (TNFa, IL-6, IL-1b) is strongly increased, whereas the expression of M2 markers such as CD206, Arg-1 and IL-10 was significantly suppressed. Consistent results have been obtained treating bone marrow-derived macrophages with hemolytic RBCs, free heme and Fe-NTA. Importantly, the addition of the heme scavenger hemopexin and the iron carrier transferrin or the chelator deferoxamine fully abolish the ability of free heme and iron to trigger M1 polarization. On the contrary, RBC transfusions in mice shape macrophages towards an M2-like anti-inflammatory phenotype. After three transfusions, serum iron and hepcidin levels significantly rise, and tissues as well as macrophages are heavily iron loaded. Macrophages show a drastic suppression of M1 markers and inflammatory cytokines, and induction of M2 markers. Interestingly, repeated transfusions result in extensive macrophage cell 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 erythropagocytosis, 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 administration 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.

This project was funded by the Federal Ministry of Education and Research, grant number: 01KG1405

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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.

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**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 Hba1c/β0 (genetically engineered hemoglobin) expression at 6 months (ASH, 2016). In an effort to optimize the proportion of patients able to discontinue blood transfusions to achieve “transfusion independence” in all patients and increase unsupported Hb levels after treatment, the manufacturing process for LentiGlobin DP was modified to increase the DP VCN and the proportion of genetically modified cells. Northstar-2 (HGB-207) is a recently initiated phase 3 study using this new manufacturing process in patients with TDT and a non-β0/β0 genotype.

**Aims:** To evaluate safety and efficacy of autologous HCT with LentiGlobin DP in patients with TDT and a non-β0/β0 genotype.

**Methods:** After providing informed consent, patients 12 to 50 years of age (N=15) will have CD34+ cells collected via mobilization and apheresis. After individualized DP manufacture and satisfaction of release criteria, the patient will receive myeloablative conditioning with single-agent busulfan (starting dose 32 mg/kg/day for 4 days, with target AUC 4500 [range 4000–5000] µM*min) followed by infusion of LentiGlobin DP. Patients will be followed for engraftment, safety and efficacy endpoints for 2 years after infusion; patients will then have the option to enroll in a 13-year follow-up study. The primary endpoint is the proportion of patients who achieve transfusion independence after DP infusion, defined as total Hb ≥9g/dL without RBC transfusions for a continuous period of ≥12 months. Secondary endpoints include time to neutrophil engraftment, adverse events, and biological parameters including VCN in peripheral blood and levels of Hba1c/β0 over time.

**Results:** As of March 1, 2017, two 20-year-old females with β0/β0 genotypes have been treated with LentiGlobin DP in the Northstar-2 trial. The DP VCN was 2.9 and 2.4 copies per diploid genome, respectively. Outcomes in all evaluable patients will be presented.

**Summary/Conclusions:** Results from the Northstar-2 study will provide data on safety and demonstrate the extent to which an increase in LentiGlobin DP VCN yields normalization of total Hb and consistently achieves transfusion independence in patients with TDT of non-β0/β0 genotypes. Optimizing DP VCN has the potential to improve outcomes across all TDT genotypes treated by investigational LentiGlobin gene therapy.

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**S815**

**CIS IS A POTENT CHECKPOINT IN NK CELL ANTI-LEUKEMIA IMMUNITY**

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**Background:** The detection of leukemia by natural killer (NK) cells is controlled...
by the integration of signals from activating and inhibitory ligands and from cytokines such as IL-15.

**Aims:** We set out to identify the negative regulators of NK cell function in order to understand why immunogenic tumours and leukaemia can evade or overcome NK cell detection and killing.

**Methods:** We used a multidisciplinary approach including RNAseq, Mass Spectrometry, functional biology, kinase enrichment and activity assays, NK cell in vitro analysis, biochemistry and de novo/experimental tumor/leukemia in vivo models.

**Results:** We identified cytokine-inducible SH2-containing protein (CIS, encoded by Cish) as a critical negative regulator of IL-15 signaling in NK cells. Cish was rapidly induced in IL-15 in response to IL-15, and deletion of Cish rendered NK cells hypersensitive to IL-15, as evidenced by enhanced proliferation, survival, IFN- 

gamma production and cytotoxicity toward tumors. This was associated with increased JAK-STAT signaling in NK cells in which Cish was deleted. Correspondingly, CISH interacted with the tyrosine kinase JAK1, inhibiting its enzymatic activity and preventing JAK phosphorylation. CISH/−/− mice are resistant to leukemia in vivo, and this was independent of MHC-I expression.

**Summary/Conclusions:** Our data uncover a potent intracellular checkpoint in NK cell-mediated tumor immunity and suggest possibilities for new cancer immunotherapies directed at blocking CIS function.

**S816**

**GENERATION OF MEMORY STEM T CELLS MODIFIED WITH A NOVEL OPTIMIZED CD30-SPECIFIC CHIMERIC ANTAGONIST RECEPTOR FOR THE TREATMENT OF CD30+ T-CELL MALIGNANCIES**

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**Background:** Peripheral T-cell lymphomas (PTCL) represent the most aggressive form among non-Hodgkin lymphomas with a very poor prognosis (5-year survival of 30%), demanding innovative novel treatment strategies. Adoptive immunotherapy with chimeric antigen receptor (CAR) engineered T cells has demonstrated its therapeutic potential in advanced hematological malignancies. However, its application to PTCL remains a formidable challenge mainly due to a lack of truly tumor-specific antigens that are not expressed on normal T cells. Anaplastic large T-cell lymphomas (ALCL) and several other subtypes of PTCL express CD30, which is expressed by activated normal T cells but no other healthy tissues. Indeed, brentuximab vedotin, an anti-CD30 antibody-drug conjugate, has shown some clinical efficacy in PTCL and ALCL patients although duration of responses is short in the majority of cases. Here, we developed a refined CD30-CAR-T cell-targeting approach, termed CARpent in vivo (in vivo CARpent) as a potential novel therapeutic strategy. We selected a novel targeting domain that is unaffected by soluble CD30 protein to prevent blockade of the CD30-CAR in vivo. Moreover, we optimized the therapy by using memory stem T cells (TSCM) to promote engraftment and persistence of CD30-CAR T cells, and we have included an EGFR 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- 


generated by depleting TSCM with an anti-EGFR monoclonal antibody. The presence of EGFR-APO2L in TSCM was verified by flow cytometry.

**Methods:** Second generation CD30-41BBL-EGFR1 CAR was generated using a scFv that recognizes a tumor-cell membrane-proximal epitope of CD30 protein (Nagata S et al. Clin Cancer Res, 2002). Naïve T cells from healthy donors were activated with anti-CD3/CD28 beads in presence of IL-7, IL-15 and IL-2 and cultured for 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 10% to 10, 1, 1.1 and 1.5 effect/target (E/T) ratios, and the tumor cell death was determined by flow cytometry. Cytokines (IFN-γ and IL-2) were analysed at 24 hours in a 5:1 E/T ratio using Luminter technology.

**Results:** The most prevalent T-cell subset at day 10 of culture, representing 84 ± 3.1% of total cells, and the CD30-CAR expression in these cells was 76.9 ± 1.0% in CD4+ TSCM and 77.3 ± 2.0% in CD8+ TSCM. Although CD30 protein was detected in a fraction of activated T cells in culture (CD4+ T cells: 32.4 ± 2.1%; CD8+ T cells: 59 ± 4.3%), lentiviral transduction of TSCM with our CD30-CAR did not compromise their ex vivo expansion (CD4+ CD30-CAR TSCM: 96.0 ± 3.2 fold expansion; CD8+ CD30-CAR TSCM: 109.0 ± 4.2 fold expansion). CD4+ CD30-CAR TSCM conferred specific cytolytic activity and lytic index (tumor cell death: 1.1 ratio: 92.6 ± 2.4% vs 0% with untransduced TSCM, P<0.001), while control CD30- target cells (Raaji) were not recognized. In addition, CD30-CAR TSCM secreted IFN-γ and IL-2 after stimulation with Karpas 299 cells (IFN-γ: 126.6 ± 18.12 pg/ml, p=0.002, IL-2: 20.47 ± 2.3 pg/ml, p=0.06 ± 0.24 pg/ml, p=0.002).

**Summary/Conclusions:** Collectively, our data demonstrate the potential to generate CD30-CAR T cells with enhanced functional attributes against CD30+ PTCL. TSCM cells can be efficiently transduced and ex vivo expanded with a novel CD30-CAR and confer potent antitumor efficacy against CD30+ PTCL in vivo. Our findings suggest the potential to improve outcome of patients with CD30+ PTCL through adoptive therapy with CD30-CAR modified T cells.

**S817**

**MESENCHYMAL STROMAL CELLS FOR THE TREATMENT OF STEROID-RESISTANT ACUTE GRAFT VERSUS HOST DISEASE: FACTORS INFLUENCING CLINICAL RESPONSES**


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**Background:** The immunosuppressive activity of mesenchymal stromal cells (MSCs) has been extensively tested for the treatment of steroid-resistant acute graft versus host disease (aGVHD). However, the factors affecting clinical responses are poorly understood.

**Aims:** We assessed the impact of MSC treatment on clinical outcomes and investigate factors influencing the response to MSCs.

**Methods:** Data collected from a cohort of 60 patients treated with MSC between May 2008 and December 2014 in the UK were analyzed. Clinical grade MSC were generated from bone marrow aspirates collected from the iliac crest of healthy donors and expanded using platelet lysate. All patients received MSC for the treatment of steroid-resistant aGVHD, defined as failure to respond to high-dose steroids (2mg/kg methyl-prednisolone) after 6 days. Informed consent was obtained from all patients in accordance with the local ethics committee requirements. Clinical responses to MSC were assessed 1 week after MSC infusion. Patients were defined as: a) Responders when an improvement of at least 50% in at least one organ affected by aGVHD was observed, or b) Non-Responders if they had stable or progressive disease.

**Results:** Patient characteristics are summarized in Table 1.

**Table 1.**

aGVHD was biopsy proven in 45 patients, while in the remaining patients the diagnosis was clinical and based on the exclusion of alternative causes. 10, 16 and 1 patients had skin, gut and liver involvement only, respectively. 16 patients exhibited gut and skin, 11 skin, gut and liver, 3 skin and liver and 3 gut and liver. 34 patients received 1 dose, while 19, 6 and 1 were treated with two, three and four doses, respectively. No side effects were observed. 36 patients (60%) responded to MSC. Amongst patients who received multiples doses (26), subsequent doses did not change the status after the first dose (24 responded, 1 did not respond), except from one patient who, although respond-
ing to the first dose, failed to respond to the second one. When we evaluated potential factors for response, organ involvement, age at transplant and the cumulative dose of MSC infused were found statistically significant. Response rate was 67% among patients with involvement of gut, skin or both, but only 22% among those with involvement of the liver (alone or in combination with skin and/or gut). Patients younger than 20 years fared better, with 88% of them responding. Conversely, only 30% and 42% of those aged 20-50 years or older than 50 responded, respectively. Lastly, higher cumulative MSC dose (>3.0x10^6/Kg) was associated with a response in 76%, while none of those receiving less than 1.5x10^6/Kg responded. All 3 factors remained significant in multivariate logistic regression analysis. Patient gender, pre-MSC therapy, interval from transplant or aGvHD diagnosis to MSC treatment and grade of aGvHD did not affect response. The impact of achieving a response 1 week after MSC had a profound impact on the overall survival at 18 months accounting for 59% in responders and 17% in non-responders (log-rank test, p<.001).

Summary/Conclusions: In our cohort of patients, MSC treatment was safe and well tolerated. We conclude that the presence of a response at one week highly impacted on the survival of patients with an otherwise very poor prognosis. Importantly, younger age at the transplant, absence of liver aGvHD involvement and use of higher MSC doses were strong predictors of a response.

S818 CARD9 CONTROLS DECTIN-1-INDUCED T-CELL CYTOTOXICITY AND TUMOR GROWTH IN MICE
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Background: Activation of the C-type lectin receptor Dectin-1 by beta-glucans triggers multiple signals within dendritic cells (DCs) that result in activation of innate immunity. While these mechanisms can potently prime CD8+ cytotoxic T cell (CTL) responses without additional adjuvants, the Dectin-1 effector pathways that control CTL induction remain unclear.

Aims: Aims of this study were: To define details of the intracellular signalling pathway responsible for cross-priming of a CTL response after activation of the C-type lectin receptor Dectin-1. To analyze whether identified signalling molecules were indispensable for antitumor immunity. To analyze whether NK cells played a role in antitumor immunity after Dectin-1-mediated CTL induction.

Methods: We used in vitro coculture between DCs (wildtype vs gene deficient) and CD8 T cells to define signalling components of Dectin-1 induced CTL cross-priming. We used WT and gene-deficient mice to define the signalling pathway of Dectin-1 induced CTL crosspriming in vivo and to test the role of this pathway for antitumor immunity by challenging mice with B16-Ova tumor cells intravenously, with or without depletion of CD8 T cells or NK cells, respectively.

Results: Here we demonstrate that Dectin-1-induced CTL cross-priming in mice does not require inflammasome activation but strictly depends on the adapter protein Card9 in vitro. In vivo, Dectin-1-mediated Card9 activation after vaccination drives both expansion and activation of antigen-specific CTLs, resulting in long-lasting CTL responses which are sufficient to protect mice from tumor challenge. This Dectin-1-induced antitumor immune response was independent of natural killer (NK) cell function and completely abrogated in Card9-deficient mice. Thus, our results demonstrate that Dectin-1-triggered Card9 signaling but not inflammasome activation can potently cross-prime antigen specific CTLs, suggesting that this pathway would be a candidate for immunotherapy and vaccine development (Figure 1).

Summary/Conclusions: We identify Card9 as central regulator of Dectin-1-induced cross-priming of cytotoxic T cells (CTLs) in mice. These antigen specific CTLs mediate potent antitumor immunity independent of inflammasome activity and NK cells. This pathway is a candidate for immunotherapy and vaccine development.
Acute lymphoblastic leukemia - Biology

E819 PRECLINICAL COMBINATION OF A NOVEL IRE1 RNASE INHIBITOR MKC-8866 AND TYROSINE KINASE INHIBITION ACTS SYNERGISTIC IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The role of the Unfolded Protein Response (UPR) in BCR-ABL1+ ALL is not properly defined yet. Although this role has never been confirmed in BCR-ABL1+ cells. To shed light on this aspect we co-cultured either SUP-B15 or TOM-1 cells with tertMSCs to test the chemo-protective effect of bone marrow stromal cells (BMMSCs) on leukemia cells. 2) We tested whether genetic knock-down of XBP1 could sensitize cells towards the effect of Imatinib and Nilotinib. To this end, primary murine pre-B cells from conditional XBP1fl/+mice, were transduced with BCR-ABL1 construct, and with either inducible cre or empty vector.

Results: IRE1 inhibitor MKC-8866 (MKC) in combination with either Imatinib (IM) or Nilotinib (NL) showed enhanced capacity to arrest proliferation and to induce cell death in BCR-ABL1+ ALL cell lines compared to single treatments, after 3 days incubation (Viable SUP-B15: MKC 30µM 94.9%±0.1, IM 10µM 78.4±0.4, Combination 78.7±0.4, MKC 30µM 94.1±0.07, NL 5µM 64.2±0.6, Combination 78.4±0.4, TOM-1: MKC 30µM 94.1±0.07, IM 10µM 89.9±0.4, Combination 78.4±0.4, Combination 17.0±1.4; MKC 30µM 94.1±0.07, NL 5µM 64.2±0.6, Combination 78.4±0.4).

Summary/Conclusions: Overall, our data demonstrate that simultaneous inhibition of IRE1 and BCR-ABL1 in BCR-ABL1+ ALL cells is a promising therapeutic strategy involving UPR inhibition that possesses translational potential and clinical application of a combined targeted therapy.

E820 HIGH-THROUGHPUT COPY NUMBER PROFILING IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA USING MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION IN COMBINATION WITH NEXT-GENERATION SEQUENCING

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Background: Development, progression and resistance of pediatric acute lymphoblastic leukemia (pALL) are widely associated with recurrent copy number abnormalities (CNAs). Multiplex ligation-dependent probe amplification (MLPA) is an established technique to screen CNAs, thus providing valuable information for risk assessment in pALL; however, the number of simultaneously analyzable genomic loci is limited to 55-60.

Aims: To introduce and test a high-throughput, high-resolution and comprehensive disease-relevant CNA profiling approach applicable to all subtypes of pALL.

Methods: A novel digitalMLPA (dMLPA) technique has been developed which combines the advantages of MLPA and next-generation sequencing (NGS), massively improving the number of genomic targets that can be analyzed for their copy number in a single reaction. Bone marrow samples from 58 patients with pALL were analyzed using this novel assay targeting ~470 genomic loci. Since copy number estimates in pre-B and pre-T ALL, respectively, followed by CDKN2A/B, PAX5, RB1, VPREB1, MLLT3, CD200/BLA, MLBX1R1, IKZF1, CASP8AP2, PTPN11, RUNX1, BTG1, TP53, IKZF3, EZH2, NFI, NKG2C, RAG2 and the PAR region for CNAs in pre-B-ALL cases and PTEN, MLLT3, FLT3, PTM2, PHF6, LEP1, CASP8AP2, PTPN11, RAS, TP53 in pre-T-ALL cases. The CNA fusions were also observed in T-ALL cases while in one BCR-ABL1+ pre-B-ALL patient, the copy number profile correctly indicated the presence of an extra Ph-chromosome. dMLPA results showed a congruency of 99.3% with those of MLPA mixes containing probes with different ligation sites for a subset of the genes. The increased resolution of dMLPA (i) allowed the detection of subclonal aberrations with an improved efficacy and confidence as compared to conventional MLPA and (ii) enabled a more patient-specific characterization of CNAs, e.g. by revealing 15 different deletion patterns across 23 samples harboring del(Php). In addition to genomic lesions specifically influencing putative or proven disease-relevant genes, 24 structural and 134 whole chromosome aberrations were detected genome-wide which was strongly facilitated by the inclusion of ~200 digital karyotyping probes covering each chromosome arm.

Summary/Conclusions: A novel NGS-based method has successfully been introduced for high-resolution profiling of CNAs in pALL. dMLPA is a robust, fast and cost-effective technique; its input DNA requirement (~20ng) is similar to those of other low-input NGS protocols and lower than the requirement for MLPA. Due to its targeted approach, data analysis is computationally less demanding as compared to most NGS methods. The number of genomic sites analyzed in our study was an order of magnitude higher than that achievable by revealing 15 different deletion patterns across 23 samples harboring del(Php). In addition to genomic lesions specifically influencing putative or proven disease-relevant genes, 24 structural and 134 whole chromosome aberrations were detected genome-wide which was strongly facilitated by the inclusion of ~200 digital karyotyping probes covering each chromosome arm.

E821 CRITICAL ROLE FOR NOTCH SIGNALLING IN B-CELL PRECURITY ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) DRUG RESISTANCE
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Background: B-cell precursor acute lymphoblastic leukemia (B-ALL) is the leading cause of cancer-related death in children and young adults. There is still a need of more efficient therapies for the subset of refractory patients. Our group has previously shown that Notch-3 and Notch-4 promote human B-ALL cell survival in presence of stromal cell support. However, the prognosis value of Notch signalling as well as its contribution in vitro and in vivo to chemotherapy has not yet been investigated.

Aims: In this study we used B-ALL cell lines and samples from new diagnosed B-ALL patients to analyse the contribution of Notch signalling to B-ALL pathogenesis in terms of prognosis, proliferation survival and drug response in vitro and in mice xenograft models of B-ALL.

Methods: B-ALL cell lines were obtained from ATCC, while B-ALL primary cells were obtained from bone marrow or peripheral blood of 30 B-ALL patients. Flow cytometry and western immunoblotting were used to study the expression of Notch receptors and ligands. Drugs used were Cytarabine (Ara-C), Dexamethasone (Dexa) and Doxorubicin (Doxo) alone or in combination with Notch modulators including anti-Notch blocking antibodies, gamma secretase inhibitors (GSis), and Notch transcription factor inhibitor (SAHM1). Mouse xenograft model of B-ALL were obtained by injecting the B-ALL line RS4;11 in...
NOD/Shi-scid/Ig/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 Doxo, revealed a reversion of the down-modulation 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 or Doxo towards B-ALL. Finally, in vivo, Notch inhibitors significantly reduced the administration to mice of a pan Notch inhibitor, i.e. the GSI XII, significantly lowered the CD19+ leukaemic burden in the bone marrow of recipient mice, potentiating anti-leukemic effect of Ara-C.

Summary/Conclusions: In this study we used both in vitro and in vivo assays to highlight the prognostic value of Notch expression in B-ALL, as well as its critical role in B-ALL cell survival and response to chemotherapy. We also demonstrated that Notch inhibitors were able to improve Ara-C-mediated reduction of blast cells in bone marrow, revealing that Notch signalling is a possible therapeutic strategy to eradicate minimal residual disease in B-ALL.

E822

REGULATION OF NOTCH AND WNT SIGNALING PATHWAYS BY NRARP IN T-CELL ACUTE LYMPHOBlastic LEUKEMIA

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Background: T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy. Although the outcome of T-ALL patients has improved over recent years, the poor prognosis of patients with resistant or relapsed disease is still a major concern. Even though NOTCH is a known driver in T-ALL, its inhibition cannot be efficiently achieved with the drugs currently available, due to their weak therapeutic effects and severe toxicity. We have shown that loss of NRARP (the wild type 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 we observed a down-regulation of NRARP expression 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. Interestingly, NRARP overexpression blocks the expansion of the T-ALL cell lines that display NOTCH1-activating mutations but promotes the expansion of the T-ALL cells without NOTCH1 mutations. Although in both cell types (WT and NOTCH1-mutated) NRARP overexpression blocks NOTCH signaling, in NOTCH1-WT T-ALL cells we observed an increase in c-Myc expression consistent with these results. T-cell-activating mutations in NOTCH1-WT NRARP overexpressing cells are more sensitive to JQ1, a small-molecule bromodomain inhibitor that targets c-Myc. NRARP is known to be positively regulated by LEF1, a DNA binding transcription factor acting downstream of WNT. Thus we sought to investigate the impact of this Lef1 in NOTCH1 signaling pathway. Very interestingly, our results show that in NOTCH1-mutant cells NRARP overexpression results in the down-regulation of the WNT signaling pathway while in NOTCH1-WT T-ALL cells results in its up-regulation.

Summary/Conclusions: Taken together our results suggest that NRARP may play a dual role in T-ALL pathogenesis, regulating both NOTCH and WNT pathways, with opposite functional effects on leukemia cells depending on NOTCH mutational status and signaling levels. This dual role may have important biological and therapeutical 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 immunophenotyping of 573 childhood B-cell precursor ALL (B-ALL), we identified eight cases with this immunophenotype among 8 “B-other” ALL (B-ALL cases negative for hyperdiploidy, ETV6/RUNX1, TCF3/PPX1 and BCR/ABL fusion genes and KMT2A rearrangements).

Aims: We aimed to characterize their genetic and biological background, to reveal to what extent they resemble ETV6/RUNX1-positive ALL and to elucidate whether they belong to the recently described ETV6/RUNX1-like ALL (Liljebäck et al., Nature Communications 2016).

Methods: We utilized microarrays to study the gene expression profile (GEP) and biological similarity of the B-ALL subtypes. Five ETV6/RUNX1-positive and five hyperdiploid ALL cases were analyzed using microarrays in parallel to seven CD27+/CD44low B-ALL cell lines. 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-positive, TCF3/PPX1-positive, KMT2A-rearranged, hyperdiploid and B-other ALL cases) whose specimens were analyzed using the same microarray. To study the genomic background, we performed comprehensive profiling using single nucleotide polymorphism (SNP) arrays and whole exome and whole transcriptome sequencing (WES and RNAseq).

Results: In the hierarchical clustering based on GEP all five ETV6/RUNX1-positive cases and 5 of 7 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 transcribed each in one patient. The most important findings are summarized in Figure 1. All 5 ETV6/RUNX1-like cases harbored a deletion of the ETV6 gene, resulting in an in-frame ETV6/BORC5 fusion in one of them. The deletion of ARP2/21 was found in 3 cases, and the deletions of PAX5, A1TP10A, BTG1 and the gain of RUNX1 were found in 2 cases each. The ARP2/21 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-involved out-of-frame fusion were each found in one case. The other cases with available material. Microarray data and the TCF3/PPX1 fusion (the former co-occurring with two activating JAK2 mutations) were found in 2 CD27+/CD44low-neg B-other cases that were closer to other B-ALL subtypes than to ETV6/RUNX1-positive ALL as indicated by GEP.

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 ARP2/21 to contribute to the specific genomic profile of ETV6/RUNX1-like ALL in addition to lesions of ETV6

Figure 1.
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 overexpression of target genes in pediatric T-ALL in Taiwan and assess 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, ETV6, CALM-AF10 and CALM-AF10 transcripts were detected by RT-PCR assays. RO-PCR with TagMan 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 LYL1 was defined as NCN > the upper limits of the 50 normal bone marrow controls. Mutations of NOTCH1, FBXW7, PHF6, JAK1, JAK2, RUNX1, WT1, NRAS, and KRAS genes were analyzed by PCR-based assays followed by direct sequencing. P16 deletion was determined by RO-PCR or multiplex ligase probe amplification (MLPA), PTEN and PHF6 deletions, MYB duplication and NCN of mutations of candidates around well-known tumour suppressors, indicating the framework analysed contains copy-numbers from tumour samples matched to normal blood samples or normal tissue from the same donor. To validate the essentiality of genes in the discovered regions we used pooled CRISPR/Cas9 editing in ALL cells with and without a deletion of the driving tumour suppressor. Results: Our framework identified several regions with potential essential genes around well-known tumour suppressors. The strongest signals in the data set were located around the tumour suppressor CKDN2A. Downstream analysis with pooled CRISPR/Cas9 editing in ALL cells with and without a CKDN2A deletion provided evidence for the essentiality of several genes in the identified region, including one gene that was essential only in CKDN2A-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 responses are observed in adolescents and adults (less than 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 exchange in numerous protein substrates.

Aims: To investigate the potential of targeting the TXN antioxidant enzymes as a novel pro-oxidative strategy in B-ALL treatment.

Methods: We have used three different cell lines representing distinct cytogenetic subgroups of B-ALL: BV-173 (BCR-ABL), SEMK-2 (MLL-AF4) and NALM-6 (t(4;14) [E2A-PBX1]). ROS levels were measured using DCFDA, RNA and protein levels of TXN-family enzymes were measured by quantitative PCR and immunoblotting, respectively. Downregulation of PRDX1 was established by a novel CRISPR/Cas9 gene editing system. We have employed lenti-
CRISPR v2 plasmid to produce lentiviral vectors encoding PRDX1-specific sgRNA and Cas-9 and used them to generate BV-173 cells with PRDX1 genomic deletion. Proliferation rate was evaluated by trypan blue exclusion method. Cytostatic/cytotoxic effects of TXN-family enzymes inhibitors, such as adenothin (ADE), auranofin (AUR) and SK053 were assessed by MTT viability assay and by detection of propidium iodide-positive cells in flow cytometry.

Figure 1. Aims: To define the biological significance of IGF2BP1 overexpression in t(12;21)(p13;q22) ET6V/RUNX1-positive ALL.

Results: We have found that B-ALL cell lines exhibit significantly higher levels of ROS as compared to normal B cells isolated from human tonsils (Fig.1A). In accordance with this observation, our analysis of TXN antioxidant enzymes gene expression in B-ALL cell lines showed their upregulation (Fig.1B). Analysis of deposited data revealed that PRDX1 expression level is the highest in B-ALL among the other types of leukemia (Fig.1C). Moreover, we have observed elevated expression of PRDX1 in malignant lymphoblasts derived from pediatric patients at both RNA and protein levels. Genomic deletion of PRDX1 in BV-173 cells leads to suppression of their proliferation rate, comparing to parental cells and cells transduced with mammalian non-targeting sgRNA. These results allow us to suspect that PRDX1 may play growth-supporting role in these cells. Targeting TXN-family enzymes was also performed with the use of various small molecule inhibitors. Both B-ALL cell lines and primary cells are sensitive to PRDX and TXN inhibitors, which reduce cell viability in dose-dependent manner.

Summary/Conclusions: All the above results suggest that targeting TXN antioxidant system may exert desirable anticancer effects in the treatment of B-ALL. Inhibitors of TXN-family enzymes can be considered as putative agents to use in combination with classical drugs and improve existing therapeutic approaches. Further studies are underway.

Methods: In this study we have used stable sublines with downregulated IGF2BP1 from our previously published study (Stoskus, Valikevičiene et al. 2016). Dynamics of viable cell population was assessed by flow cytometry using 7-AAD staining (BD Biosciences) following 72 hrs culture in complete medium. An EdU flow assay (Thermo Fisher Scientific, TFS) was used to assay DNA replication in proliferating cells. Spontaneous and doxorubicin (Doxo), staurosporine (STS), and STAT3 selective inhibitor S3i-201 (all from Santa Cruz Biotechnology) induced cell death rates were determined by Annexin V (TFS) and 7-AAD staining. All samples were analyzed on Accuri C6 cytometer (Accuri Cytometers) using CFlow Plus and FCS Express software (De Novo Software). IGF2BP1, ET6V/RUNX1, and STAT3 RT-qPCR was performed essentially as reported previously (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 (r=0.8253, p<0.001, slope 0.4959) and also STAT3 transcript levels (r=0.7709, p=0.002, slope 0.6436). These data suggest that STAT3 transcript is also a potentially regulated by RNA-binding protein IGF2BP1 in t(12;21)(p13;q22)-positive ALL model cells (Fig 1).

Figure 1.

Summary/Conclusions: We provide evidence that IGF2BP1 promotes survival of t(12;21)(p13;q22)-positive ALL model cells through cell cycle progression and preventing spontaneous cell death. Potentiation of ET6V/RUNX1®STAT3 signaling axis is one of the possible mechanisms responsible for this phenotype as IGF2BP1 maintains appropriate levels of primarily ET6V/RUNX1 and also STAT3 mRNAs. Further studies are clearly warranted to further delineate the role of IGF2BP1 in t(12;21)(p13;q22)-positive ALL (Stoskus, Eidukaite et al. 2016).

E829

6-MERCAPTOPURINE PROMOTES ENERGETIC FAILURE IN LEUKEMIC T-CELL LINE JURKAT

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Background: 6-Mercaptopurine (6-MP) is a thiopurine drug with antiproliferative effects by blocking purine synthesis. 6-MP is largely prescribed for the treatment of childhood acute lymphoblastic leukemia (ALL). Recent evidence

...
suggest that 6-MP inhibits the phosphatidylinositol 3 kinase (PI3K)/ mammalian target of Rapamycin (mTOR) signaling pathway and modulates the transcriptional activity of hypoxia inducible factor 1α (HIF-1α). As mTOR and HIF-1α are key mediators of metabolic reprogramming in cancer and normal T cells, we hypothesized that 6-MP can impact cellular metabolic remodeling through its action on nucleotide synthesis. Metabolic reprogramming fosters glycolysis, glucose transporter expression, polarization of lipid metabolism, cell growth, and proliferation, a key feature of cancer cells. This metabolic switch is regulated by metabolic checkpoints, including mTOR, AMP-activated protein kinase (AMPK) and the oncogenes Myc and HIF-1α.

Aims: Our objective is to study the impact of the antiproliferative molecule 6-mercaptopurine (6-MP) on proliferating T-cell leukemia cells metabolic reprogramming and nuclear activity.

Methods: In vitro experiments were performed in a Jurkat T cell line. Cells were incubated with 6-MP from 6h to 72h. We used RT-PCR, Western Blot, glucose uptake and glycolytic and glutaminolytic flux to evaluate the metabolic effects of 6-MP.

Results: Our results showed that 6-MP reduces ATP content as early as after 2 hours of treatment and this decrease is maintained up to 72 hours. As AMPK is an energetic sensor activated with low ATP content, we studied AMPK activation after 6-MP treatment. We observed that 6-MP treatment activates AMPK after 6 and 48 hours of treatment. Moreover, 6-MP significantly modifies the transcriptome 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 OXPHOS (oxidative phosphorylation). The production of lactate, a marker of aerobic glycolysis, is significantly decreased by 30% after 6-MP treatment for 48 hours, meaning that aerobic glycolysis is also inhibited. However, 6-MP has no effect on glucose uptake or on glucose transporters (Glut1 or Glut3, SLC2A1 or SLC2A3) on the cell membrane, 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 modification 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.

**Table 1:**

<table>
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<tr>
<th>Candidate Gene</th>
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</tr>
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</table>

E830

**GENETIC ABERRATIONS IN ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA AND THEIR IMPACT ON CLINICAL OUTCOME**

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**Background:** Genetic alterations have prognostic impact on pediatric patients with B cell acute lymphoblastic leukemia (B-ALL). Genomic landscape and its impact on clinical outcome is less understood in adults with B-ALL.

**Aims:** To describe the landscape of genomic aberrations and analyze the correlation with clinical characteristics and prognostic impact in adults with B-ALL.

**Methods:** We assessed bone marrow specimens from 64 consecutive adults with a median age of 51 years (range 18 to 80) with previously untreated B-ALL between 2012 and 2015. The cohort included 23 Philadelphia chromosome (ph)-positive and 41 Ph-negative B-ALL. Sixty patients (94%) were treated with hyper-CVAD (hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone alternating with high-dose methotrexate and cytarabine)-based regimen; 23 of them received additional tyrosine kinase inhibitor for Ph-positive disease (dasatinib [N=4] or ponatinib [N=19]). Four patients (6%) were treated with augmented BFM (Berlin-Frankfurt-Munster) regimen. Genomic DNA extracts were sequenced by targeted capture exome sequencing of 295 genes that are recurrently mutated in hematologic malignancies (median 280x coverage). The panel included more than 1000 cytoSNP position evenly distributed among the genome that allowed estimation of copy number variations (CNVs). Point mutations were called by Mutect/Pindel algorithm.

**Results:** Among the 64 patients, we detected 70 point mutations in 40 genes in 38 patients (54%). Ph-positive ALL had significantly less point mutations than Ph-negative ALL (median number of mutations/patient 0 [range: 0-2] versus 1 [range: 0-8], P=0.002). The most frequently mutated genes were TP53 (17%) followed by MLLT3 (12%), ARID1A (5%), NRAS (5%), NF1 (5%), RUNXI (5%), and TET2 (5%). TP53 mutations were strongly associated with Ph-negative B-ALL (P=0.004) and low hypothyroidism (P=0.009). Recurrent CNVs involved loss/deletion in genes such as PAX5 (28%), TCF3 (38%), IKZF1 (31%), CDKN2A/2B (31%), BTLA (25%), CD200 (22%), ETX2 (22%), RBL1 (20%), MIB1/FAK1/ER4 (15%), ERG (14%), and MLT7 (11%), whereas gain/amplification was detected in NR3C2 (18%), ERG (15%), RUNXI (15%), and LEF1 (14%). MLT7 loss/del was specific to Ph-negative ALL (0% for Ph-positive versus 17% for Ph-negative, P=0.036) and MIR15-16/1-1 deletion/Deletion had non-statistically significant association with Ph- ALL (4% versus 22% respectively, P=0.06). In this cohort, 78% and 100% of the Ph-negative and Ph-positive ALL achieved complete remission, respectively. None of the point mutations or CNVs were associated with differential response to therapy. Survival analysis was stratified by Ph status. Complex karyotype had trend toward worse event-free survival (EFS) (median EFS 3.6 months versus 26.3 months, P=0.06) in Ph-negative ALL. None of the point mutations or CNVs was associated with EFS/overall survival (OS) in Ph-negative ALL. Notably, TP53 mutation nor low hypothyroidid did not affect EFS/OS in the current cohort. In Ph-positive ALL, IKZF1 deletion/loss was associated with a trend toward worse EFS (median EFS 3.6 months versus 21.3 months, P=0.07) but it did not affect OS.

**Summary/Conclusions:** Genetic analysis highlights the molecular heterogeneity of adult B-ALL. Adult B-ALL is frequently associated with CNVs and point mutations are less frequent. Prognostic impact of genetic alteration in adult B-ALL appears to be limited except for IKZF1 deletion/loss, which may predict worse EFS in Ph-positive B-ALL.

E831

**PROFILING OF RECURRENT COPY NUMBER ALTERATIONS IN RELAPSED ADULT B CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKAEMIA**

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**Background:** The survival rate of relapsed adult acute lymphoblastic leukaemia (ALL) is around 10%. Aims: We looked for recurrent Copy Number Alterations (CNA) in relapsed adult B cell progenitor ALL (BCP-ALL) to shed light into the molecular mechanisms of relapse.

**Methods:** BM or PB samples with at least 30% of blasts from 31 adult BCP-ALL patients at 1st relapse and, of them, 21 paired diagnosis and relapse samples were analysed by MLPA (MRC-Holland, The Netherlands). 19 out of these 21 paired samples were analysed by SNP array with CytoScan HD chips (Affymetrix, Santa Clara, California, USA) with true CNA. The analysis compassed a minimum of 25 markers, and 25 markers and 320Mbs for CN-LOH.

**Table 1:**

<table>
<thead>
<tr>
<th>Candidate Gene</th>
<th>Mutation Type</th>
<th>Exon</th>
<th>Non-synonymous Codon</th>
<th>Allele Frequency</th>
</tr>
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<tbody>
<tr>
<td>ABCB1</td>
<td>Missense</td>
<td>22</td>
<td>C&gt;T</td>
<td>0.01</td>
</tr>
<tr>
<td>AKT1</td>
<td>frameshift</td>
<td>3</td>
<td>Ddel</td>
<td>0.02</td>
</tr>
<tr>
<td>AURK1</td>
<td>deletion</td>
<td>5</td>
<td>Ddel</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Results: With a median follow up of 12.43 [2.4;3.0] months, the median OS of the 31 patients at first relapse was 7.9 months, [2.4;13.8]. 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], 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%). 8 out of 9 heterozygous CDKN2A/B deletions, homozygous deletions were more frequent at relapse (from 8 heterozygous CDKN2A/B deleted patients at diagnosis, 7 became homozygous at relapse, p=0.070). SNP arrays detected 554 CNA (409 DEL, 125 DUP and 20 LOH) in 34 samples of 19 patients. At diagnosis (n=16 patients) the mean number of CNA was 12.5 (9.8 DEL, 2.3 DUP and 0.4 LOH), while at first relapse (n=13 patients) was 17.8 CNA (12.6 DEL, 4.2 DUP and 1 LOH) and in second relapse (n=5 patients) was 21 CNA (14.6 DEL, 6.4 DUP and 0 LOH)(p=0.007). All matched diagnosis and first relapse samples (available for 10 patients) showed common CNA. In 6/10 cases some of CNA were retained from diagnosis while others were acquired or lost at relapse (suggesting a clonal development of the leukemic clone). In the remaining 4/10 diagnosis CNA were retained and 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 22q duplications and/or small 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 strategies, not only on their molecular targets. Finally, we hope that Instituto de Salud Carlos III, Ministerio de Economía y Competividad, Spain, Red Temática de Investigación Cooperativa en Cáncer (RTICC, FEDER) (RD12/0036/0044); Sociedad Española Hematología y Hemoterapia; 2014 SGR225 (GRE) Generalitat de Catalunya; Fundació Internacional Josep Carreras, Celgene Spain and “la Caixa” Foundation.

E832
IGF1/IR/IRS PHARMACOLOGICAL INHIBITION REDUCES CELL PROLIFERATION AND MIGRATION IN ACUTE LYMPHOBLASTIC LEUKEMIA CELLS
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1Internal Medicine, University of Sao Paulo at Ribeirao Preto Medical School, Ribeirao Preto, Brazil, 2Randall Division of Cell and Molecular Biophysics, King's College London, London, United Kingdom

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 (IGF1R), and the subsequent activation of its substrates, IRS1 and IRS2, which transmit mitogenic and antiapoptotic signals, mainly through the modulation of the PI3K/AKT/mTOR and MAPK signaling pathways. These signaling pathways play an important function in cell proliferation, survival and migration of leukemia cells. We have previously noticed that NT157 (IRS1/2 pharmacological inhibitor) significantly decreased cell viability and induced apoptosis in T-ALL (Jurkat and MOLT-4), and in B-ALL (Namalwa and Raji) cells and in primary ALL cells (T-ALL [n=2] and B-ALL [n=2]), although did not presented citotoxicity in peripheral blood mononuclear cells (PBMC) from healthy donors. In addition, NT157 was able to induce the p21 (CDK1WA1) expression, which is a cell cycle arrest-related gene. We also observed that OSI-906 (IGF1/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 is not well understood.

Aims: We herein aimed to investigate the impact of the pharmacological IGF1/IR and IRS1/2 inhibition on cell proliferation and migration in ALL cells.

Methods: T-ALL Jurkat and B-ALL Namalwa were used. Cell lines were treated or not with NT157 at 0.2, 0.4, 0.8, 1.6 and 3.2 µM, or with OSI-906 at 0.5, 1, 5 and 10 µM. After 24 hours 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 monoclonal layer). Statistical analyses were performed by the ANOVA. Value p<0.05 was considered statistically significant.

Results: In vivo and in vitro, NT157 strongly reduces cell proliferation in a dose-dependent manner (p<0.05) after 24 hours of treatment. OSI-906 was not able to reduce cell proliferation in these cell lines. The 24 hours treatment with 10µM OSI-906 decreased accumulated distance (µm) and velocity (µm/min), while 0.4µM NT157 reduces only the accumulated distance of Jurkat cells under migration assay into fibronectin monolayer, after being filtered by time-lapse microscopy for 3 hours; the images were captured every 1.5 minutes. Although there is a trend for reduction, cell adhesion between Jurkat and Namalwa leukemia cells and the human endothelial cell monolayer was not significantly modulated by treatment with both inhibitors.

Summary/Conclusions: The reduction in cell proliferation found during 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 IGF1/IR/IRS1 pathway in the break of the blood-brain barrier.

E833
LEUKEMIA-PROPELLING CELLS DEMONSTRATED DISTINCTIVE GENE EXPRESSION PROFILES COMPARED WITH THE OTHER CELL FRACTIONS IN PATIENTS WITH DE NOVO PHILADELPHIA CHROMOSOME-POSITIVE T-ALL
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Background: Relapse remains one of the major obstacles in Philadelphia chro- mosome-positive acute lymphoblastic leukemia (Ph+ALL) even after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Relapse of Ph+ALL may result in a fatal outcome, and is a key factor for determining the long-term cure of Ph+ALL patients. Despite extensive research, there is no established treatment strategy for patients with relapsed or refractory Ph+ALL. The aim of this study was to investigate the potential of peripheral blood mononuclear cells (PBMCs) from Ph+ALL patients undergoing allo-HSCT as a new source of cellular therapy. PBMCs were sorted into two populations: leukemia propagation cells (LPCs) and normal hematopoietic cells (NHCs). The expression profiles of the sorted LPCs and other cell fractions were measured using RNA sequencing (RNA-Seq) to identify potential therapeutic targets.

Methods: Twenty patients with de novo Ph+ALL were enrolled for this study at Peking University Institute of Hematology from 2015 to 2018. The LPCs (CD34+CD38-CD58-) and other cell fractions (including CD34+CD38+CD58+, CD34-CD38+CD58+ and CD34-CD38+CD58-) were sorted from the bone marrow mononuclear cells of de novo Ph+ALL patients (N=3) using a FACS Aria II. Differential expression analysis between LPCs and the other cell fractions were performed using RNA sequencing (RNA-Seq) and the DESeq package (1.10.1). Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. RNA-Seq results were partially validated by a TaqMan-based real-time quantitative polymerase chain reaction (qRT-PCR) technique. Moreover, cell cycle status was compared between LPCs and other cell fractions in de novo Ph+ALL patients by flow cytometry.

Results: 1021 genes (301 up-regulated and 720 down-regulated), 1245 genes (354 up-regulated and 891 down-regulated) and 1228 genes (248 up-regulated and 980 down-regulated) were differentially expressed between LPCs and other cell fractions in de novo Ph+ALL patients. (Patient No 1), B-ALL (Patient No 2), and Ph+ALL and Other Cells, (Patient No 3), respectively. Most of differential expression of genes (DEGs) are related to the regulation of cell cycle and metabolism. GO analysis identified enriched terms of biological functions in DEGs including ATP binding process, ribonucleotide binding process, nucleoside binding process, DNA replication process, primary metabolism process, etc. KEGG analysis showed significantly enriched signaling pathways involved in DEGs including cell cycle, DNA replication, nucleotide metabolic pathways, biosynthesis of amino acids, glutathione metabolism, p53 signaling pathway, etc. Consistent with RNA-Seq results, mRNA levels of the cell cycle-related genes, such as CDK4 and p21/WAF1, were significantly lower in LPCs fractions than those in other cell fractions. Moreover, the frequencies of quiescent cells in LPCs were significantly higher than those in other cell fractions.

Summary/Conclusions: Distinctive gene expression profiles and cluster, which are mostly related to the regulation of cell cycle and metabolism, were demon- strated between LPCs and other cell fractions in Ph+ALL patients. In addition, we were able to identify potential therapeutic targets toward LPCs in Ph+ALL. Therefore, our data indicate that it would be of value to develop LPCs biomarkers to contribute to personalized leukemia therapy and the need to identify therapeutic targets directed toward LPCs in Ph+ALL.

E834
T-CELL LEUKEMIA SENSITIVITY TO FARNESYL TRANSFERASE INHIBITION USING TIPIFARNIB
R. Mondejar1,*, R. Alonso1, C. Perez1, F. Burrows2, C. Schozl2, A. Gualberto2, M.A. Piris3
Acute lymphoblastic leukemia - Clinical

E835

HOSPITALIZATION FOR PATIENTS IN THE U.S. AND EU TREATED WITH INOTUZUMAB OZOGAMICIN VS STANDARD OF CARE FOR RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA IN A GLOBAL PHASE 3 TRIAL

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Background: Inotuzumab Ozogamicin (InO), an anti-CD22 antibody-calicheamicin conjugate, with its once a week one-hour infusion schedule, has demonstrated lower hospital utilization, in association with a clinically meaningful improvement in overall survival, high rate of complete remission, favorable patient-reported outcomes (PRO), and generally manageable safety profile versus standard of care (SOC, intensive chemotherapy) for relapsed/refractory acute lymphoblastic leukemia (R/R ALL) in the phase 3 INO-VATE trial.

Aims: This study aims to determine the regional-specific hospitalization days per patient in the INO-VATE trial.

Methods: Patients receiving study treatment (safety population) and recruited from the US and the EU were included in the analyses. The total number of days hospitalized for each patient was calculated. Hospital days prior to randomization and those after the end of study treatment were excluded. Due to different durations of treatment for InO and SOC (median 1 vs 3 cycles), calculations were reported for cycle 1 treatment period (randomization to end of cycle 1) and for the entire treatment period (all cycles - randomization to end of treatment).

Results: A total of 264 patients from the safety population of the phase 3 INO-VATE trial were available for the analyses. 149 were from the US, and 115 from 11 of the EU countries. The percentage of patients requiring hospitalization was lower for InO compared to SOC (Table). The median and mean hospitalization days were shorter for patients in the InO arm compared to the SOC arm across both regions. The difference between the two treatment arms appears to be greater in the US compared to the EU. Hospitalizations in the US appear to be shorter than in the EU, particularly for patients receiving InO.

Table 1. Hospitalizations in R/R ALL patients from the INO-VATE trial.

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<thead>
<tr>
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<th>Mean Days</th>
<th>Median Days</th>
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<tr>
<td>All-cycles US</td>
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<td>29 (25-30)</td>
</tr>
<tr>
<td>All-cycles EU</td>
<td>39 (37-41)</td>
<td>36 (32-39)</td>
</tr>
<tr>
<td>All-cycles US</td>
<td>32 (30-35)</td>
<td>29 (25-30)</td>
</tr>
<tr>
<td>All-cycles EU</td>
<td>39 (37-41)</td>
<td>36 (32-39)</td>
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</table>

Summary/Conclusions: InO treatment in R/R ALL is associated with less hospitalization across both the US and EU compared to SOC, consistent with InO’s better efficacy, tolerability, PRO and dosing schedule. The finding that US has lower hospitalization than the EU might be explained by different patient care practices in the two regions. Given that hospitalization is the biggest cost driver in cancer care, the data suggest both EU and US could benefit from cost-savings of less hospitalization with InO treatment.

E836

NON-INTENSIVE BUT NON-INTERRUPTIVE TREATMENT WITH FEWER ALLO-HSCT IS EFFECTIVE STRATEGY FOR ADULT PH-NEGATIVE B-CELL PRECURSOR (BCP-) ALL: OUTCOME OF THE RUSSIAN PROSPECTIVE MULTICENTER ALL-2009 STUDY

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1BMT department, 2Haematology department, National Research Center for Hematology, Moscow, 3BMT department, Rassia Gorbacheva Memorial Research Institute for Pediatric Oncology, Hematology and Transplantation, St. Petersburg, 4Haematology department, N.N. Blokhin Russian Cancer Research Center, 5Scientific clinical laboratory of clinical bacteriology, mycology and antibiotic therapy, 6Cytogenetics laboratory, 7Department of biostatistics, National Research Center for Hematology, Moscow, Russian Federation

Background: As Ph-negative-BCP-ALL in adults remains less favorable in prognosis than T-ALL, and by expert opinion needs intensive protocols with high portion of allo-HSCT, the results of treatment based on the different approaches are escalated but non-interruptive treatment with low numbers of allo-HSCT- may be of interest and can provide new insights to the common view.

Aims: to evaluate survival data and risk groups in Ph-neg-BCP-ALL pts in the RALL-study.
Methods: The ALL-2009 (NCT01193933) was initiated in Apr 2009. The treatment plan was identical for all risk groups with allo-HSCT indicated only for very high-risk BCP-ALL (t(4;11), t(1;19), WBC >100). Since Apr 2009 till Dec 2016, 329 Ph-negative ALL pts (m.age 28 y (15-55), f/m 147/182) were recruited. Phenotype was unknown in 6 pts, biphenotypic AL was diagnosed in 1.2% (n=4), T-ALL-LBL in 38.7% (n=125), BCP-ALL in 59.1% (n=194). Among BCP-ALL there were 54 early pre-B ALL (27.8%), 101 common-ALL (52%), 39 pre-B ALL (20.2%). In BCP-ALL pts m.age was 27 y (15-54), f/m 99/95, initial WBC >9,4*10⁹/l (0.4-899,0), LDH 901 IU (31-13059), CNS leukemia - in 17 pts (8.7%), mediastinal mass - in 3 (1.5%), splenomegaly- in 111 (57.2%). Standard cytogentic was defined in 124 pts (64%), 11 had no mitosis, so information is avail-able in 58.2% (n=115). 14.8% of BCP-ALL (n=49/113) pts had normal karyotype (NK); 7.9% (n=9) and 1.8% (n=2) - had t(4;11) and t(1;19) respectively; other abnormalities were detected in 53 (46.9%), including p53 (3.2%), +8 (6.3%), complex karyotype (7.9%), hyperdiploidy (16%), diploidy (22.2%). etc. 9 BCP-ALL patients (n=47) were not qualified by the risk in the data-base; 68.1% (n=126) were attributed to the high risk (HR) group (WBC >30; EGIL BI, LDH>2N; late CR; t(4;11)-pos). The analysis was performed in Feb 2017. 191 pts were available for induction outcomes, DFS and relapse probability (RP), and all pts – for overall survival (OS).

Results: CR rate in 191 pts was 87.4% (n=167), induction death occurred in 8.9% (n=17), resistance was registered in 3.7% (n=9). Late responders constituted 13.6% (n=26). Death in CR on chemotherapy was 6.3% (n=12) and 1 death after alloHSCT. All-HSCT was performed in 13 (6 – matched related donor, 7 MUD) of 176 patients who survived induction (74%), 11 of them – in 1CR. Totally 59 pts (34.9%) had relapsed. At 7y OS for the whole cohort con-stituted – 54.3%, DFS – 56.5%, RP – 35.4%. In a multivariate analysis for BCP-ALL among common risk factors (age >30y, initial risk group, WBC >30, LDH>2N, immunophenotype, late CR >35d, CNS leukemia, cytogenetics) age, WBC, t(4;11) became statistically significant for OS, DFS and RP. We developed a new threshold for the most valuable risk factors. New risk groups stratification demonstrated 7y OS=79%, DFS=71%, RP=23%, in the standard risk (SR) group (age <27y, WBC <7*10⁹/l, no t(4;11)) and 46%,45%,47%, respectively, in the HR group (age >27y,WBC >7*10⁹/l, t(4;11)).

E837

POST-INDUCTION MINIMAL RESIDUAL DISEASE RESPONSE DETERMINED BY MULTICOLOR FLOW CYTOMETRY IS A POWERFUL INDICATOR OF EVENT-FREE-SURVIVAL IN THE CHILDHOOD T-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 compared 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 that post-induction (Day-78) FC-MRD was more relevant in the pre-diction of 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 dexamethasone in place of prednisolone. MRD was performed using 10-color FC-MRD assay on Navios flow-cytometer (Beckman Coulter, BC) and MRD analysis was performed with Kaluza software v.1.3 (BC). Any detectable level of MRD (≥20 events) was defined as MRD-positive. Events included relapse & disease-related deaths. Statistical analysis was performed using SPSS v.16.

Results: The median age of patients was 11.5 years (range 2–16 y; M:F=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 PI-MRD-negative & 1.2% (6/58) PI-MRD-positive patients. PC-MRD was available in 64 patients (30/42 of PI-MRD-negative & 6/58 of PI-MRD-positive). PC-MRD was positive in 28% (18/64) (median, 0.2% & range, 0.009% to 4%). PI-MRD positivity was significantly high in 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-Mayer curve shown in Figure 1). Interestingly, there was no difference in EFS for PI-MRD <0.01% vs >0.01%, suggesting any level of PI-MRD positive indicates 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 in second remission is poor. Therefore, 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

Figure 1.
E839

SINGLE-AGENT MOR208 IN PATIENTS WITH RELAPSED/REFRACTORY (R/R) B-CELL ACUTE LYMPHOBlastic LEUKEMIA (B-ALL): A SINGLE-ARM PHASE II STUDY

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Background: CD19 is a type I transmembrane glycoprotein that is expressed throughout B-cell development until terminal plasma cell differentiation. CD19 is also broadly and homogeneously expressed across different B-cell malignancies, including B-ALL. MOR208 is a CD19 monoclonal antibody with an enhanced Fc region, which leads to a potentiation of antigen-dependent cell-mediated cytotoxicity and antigen-dependent cell-mediated phagocytosis. Anti-CD19 therapy can significantly improve the efficacy and safety of single-agent MOR208 in the treatment of patients with R/R B-ALL.

Methods: This is a single-arm phase II study of MOR208 in patients aged ≥16 years with histologically confirmed R/R B-ALL with progression after at least one prior therapy. Patients with Philadelphia-chromosome-positive (Ph+) B-ALL were excluded. Induction therapy was not used. Baseline target lesion was measured pre-dose. Data was collected on patient demographics, prior therapy, laboratory results, and best overall response during the treatment period.

Results: 2 patients were enrolled; median age was 21 years (range 16–79), 1 (50%) patients was male. 2 (100%) patients received the 12mg/kg IV, i.e. 1 CR and 1 PR. The major toxicities seen were neutropenia, thrombocytopenia, and hyperglycemia (each 50%). Infusion-related reactions were reported in 2 patients (10%); 1 patient received extended MOR208 (12 cycles) treatment. A further 4 (20%) patients did not have a complete response (CR) or partial response (PR) but did not discontinue treatment. The median progression-free survival (PFS) for all patients was 5 months (95% CI 3.3–9.9 months).

Conclusion: Single-agent MOR208 is well tolerated with manageable toxicity. Further studies in this population are warranted, including combination studies with other agents.

E840

UPDATED RESULTS FROM ZUMA-4: A PHASE 1/2 STUDY OF KTE-C19 CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL THERAPY IN PEDIATRIC AND ADOLESCENT PATIENTS WITH RELAPSED/REFRACTORY ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) exhibits a bimodal age distribution with 60% of cases occurring in children and adolescents (<20 y) and 25% in older adults (>45 y; http://seer.cancer.gov/csr/1975_2013/). As of 19 Jan 2017, 5 patients have enrolled and 4 have been treated with KTE-C19. 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 Cancer 2015;15:77). 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:LB-6). Here, we present updated results from the phase 1 portion of ZUMA-4, 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 2x10^6 CAR T cells/kg after low-dose conditioning chemotherapy consisting of cyclophosphamide (900mg/m² once) and fludarabine (25mg/m²/d for 3 days) (CYFlu). The primary endpoint of phase 1 is the safety of KTE-C19. 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 2x10^6 CAR T cells/kg. KTE-C19 was successfully manufactured and administered in a centralized, streamlined 6-8-day process for all patients across a 2-week period. One baseline absolute lymphocyte count (0.21–1.0x10^9/L), except for 1 patient who had disease progression with white blood cells 150,000/mL at apheresis and <0.2% T cells in the apheresis collection. All 4 treated patients had high disease burden with a median marrow lymphoblast content of 57%...
(range, 41–99%). All 4 patients received bridging chemotherapy during the manufacturing period before conditioning chemotherapy and KTE-C19. No patient experienced a dose-limiting toxicity. One patient had a grade 5 adverse event of disseminated mucormycosis which was not related to KTE-C19. Cytokine release syndrome was reported in all 4 patients (all ≤ grade 3); neurologic events were reported in 1 patient (grade 3). All cytokine release syndrome events resolved with tocilizumab, corticosteroids, and/or sil kitumab plus other supportive care with a median duration of 8.5 days (range, 4-16) days. Minimal residual disease-negative remission was observed in all 4 patients. One patient received stem cell transplant post-remission, which is allowed per protocol at investigator discretion. Peak expansion of CAR T cells occurred 1-2 weeks post-KTE-C19 infusion. Updated data with additional patients, different dose of KTE-C19, earlier tocilizumab use, and biomarkers will be presented.

Summary/Conclusions: KTE-C19 after low-dose CyFlu has been tolerable and appears safe for further analysis in pediatric and adolescent patients with R/R ALL. No toxicities were observed with KTE-C19 at the 2×10^6 cells/kg dose in patients despite high leukemic burden. All patients receiving KTE-C19 achieved a minimal residual disease-negative remission. Based on these results, ZUMA-4 continues to enroll (NCT02625480).

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: 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}, and 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.

Figure 1. Results: Total number of 103 patients was evaluated. Nine of them (8.7%) who did not reach a hematological remission on D26 were excluded from the study. The estimated mean 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.
Background: Minimal residual disease (MRD) has been proven to be the most important indicator of relapse in BCPALL. Recently, flow-cytometry based MRD has been shown to achieve a sensitivity of <10^{-5} using a standardised panel with high number of event acquisition. However, high-sensitivity BMRD analysis is based on experience and acquisition of high number of events also includes other rare BM cellular elements and artifacts. We present a study of the cost-effective high-sensitivity 10-color single tube FC-MRD assay in BCPALL along with description of rare BM cellular elements and artifacts causing interference in analysis.

Aims: 1. To study the applicability and sensitivity of a 10-color high event single tube FC-MRD assay for BCPALL; 2. To document the rare BM cellular elements and artifacts causing interference in high-sensitivity FC-MRD assay for BCPALL and describe their prevalence and immunophenotypic features.

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, CD33, CD58, CD98, CD123 and CD25/CD73 with an additional 4-color nuclear dye (SYTO13) tube. Samples with cluster of x20 and x2 leukemia associated phenotypes (LAIPs) were called MRD-positive. High number of events were acquired for MRD-assay (1.5 to 6 million). To evaluate the applicability of assay, number of LAIPs were determined in diagnostic and MRD samples. In addition, the frequency and antigen expression pattern of mimics and artifacts were studied.

Results: We studied 230 BCPALL MRD samples. High number of events was acquired for MRD-assay with median-events 3427000 (range, 1678000 to 6052800). We determined the limit of detection (LOD=10 events) and limit of quantification (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%, 0.001% - 0.01%, 0.01% - 0.1%, 0.1% - 1% and >1% and they were respectively 1.74%, 10.43%, 13.48%, 5.65% and 10.00%. Furthermore, in 24 samples with MRD-positive ≤0.01% and >1.5 million acquired-events, the results were compared between time-gated initial 500000-events, 1000000-events and all events acquired. Sixteen samples among these were found to be negative in initial 500000-events and eight in initial 1000000-events but we highlighted the importance of acquisition of >1.5 million cells. Further, we categorized different rare cellular events and artifacts in the following way: 1) CD34+ mature B cells; 2) CD10+ mature B cells; 3) CD73+ mesenchymal/stem cells and endothelial cells; 4) CD123+ CD19+ 7PD precursors; 5) CD86+ CD58+ B cell precursors (BCP); 6) CD19+ NK cells (Table 1). We also described their immunophenotypic features highlighting the differentiating features from MRD and B cell precursors (Figure 1).

Table 1.

<table>
<thead>
<tr>
<th>CD34</th>
<th>CD10</th>
<th>CD19</th>
<th>CD20</th>
<th>CD34</th>
<th>CD33</th>
<th>CD58</th>
<th>CD98</th>
<th>CD123</th>
<th>CD25/CD73</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1%</td>
<td>0.01%</td>
<td>0.1%</td>
<td>1%</td>
<td>&gt;1%</td>
<td>&lt;0.01%</td>
<td>0.001%</td>
<td>&gt;1%</td>
<td>&lt;0.01%</td>
<td>&gt;1%</td>
</tr>
</tbody>
</table>

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.
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 follows 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.

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**E845**

**NUDT15 VARIANT CONTRIBUTING TO HEMATOPOIETIC TOXICITY WITH LOW 6-TGN LEVEL IN KOREAN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

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**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 TPNMT 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 administered to 36.5, 29.9, and 21.9% of patients in LR, IR, and HR groups (p=0.05). HR exhibited significantly longer days of therapy interruption (HR 167 vs LR 30, p<0.01) and days of leukopenia (HR 131 vs LR 92 vs LR 59, p<0.01). The lowest WBC and platelet counts and hemoglobin level were observed in HR. 6-TGN level (pmole/8x10^8 RBC) divided by 6-MP dose (mg/m2) was the lowest in HR group (HR 4.4 vs LR 13.3 vs HR 14.7, p<0.01). The lowest WBC and platelet counts and hemoglobin level were observed in HR. 6-TGN level (pmole/8x10^8 RBC) divided by 6-MP dose (mg/m2) was the lowest in HR group (HR 4.4 vs LR 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.

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**E846**

**USING NEXT GENERATION SEQUENCING TO DETECT CLONAL TRG AND TRB GENE REARRANGEMENTS**

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**Background:** During early T-cell development, somatic rearrangements occur within T cell receptor beta (TRB) locus that bring together, sequentially, the known TRG and TRB gene segments of IGH, IGK, and IGJ joining regions (J) are the current gold standard for clonality testing in suspected B-cell malignancies. Recently, next-generation sequencing (NGS) based approaches for immune receptor genes have been developed that improve sensitivity and identify the specific V-(D)-J DNA sequences required to track clones in follow-up testing. We have developed comprehensive LympoTrack™ assay on both MiSeq® and PGM® platforms, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LympoTrack™ assay and MiSeq and PGM Assays to the IGH PCR-CE assay by testing in 59 anonymized, blinded clinical samples.

**Aims:** To assess the clinical performance of LympoTrack™ IGH MiSeq and PGM Assays

**Methods:** LympoTrack™ IGH Assay has been developed for both the MiSeq and PGM platforms. The analytical performance of the LympoTrack™ IGH FR master mixes were manufactured with 12 indices to allow analysis of 10 samples with 2 controls. The sequencing data was generated using Illumina® MiSeq™ platform, which detect the vast majority of rearrangements in a single NGS run. In this pilot study, we compared the performance of both LympoTrack™ IGH MiSeq and PGM Assays to the IGH PCR-CE assay by testing in 59 anonymized, blinded clinical samples.
CORRELATION BETWEEN A 10-COLOR FLOW CYTOMETRIC MINIMAL RESIDUAL DISEASE (MRD) ANALYSIS AND MOLECULAR MRD IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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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, CD71, CD20, CD52, CD10, CD25, CD34, and CD38 markers. These markers were selected to provide at least two targets for identification of B-lineage cells, and to include the most frequently aberrant markers in pre-cursor B-lineage ALL samples. Subjects were grouped according to patients’ capacity of action and reaction.

Background: Hypoglycemia has been reported as a rare side effect in children and adolescents treated for acute lymphoblastic leukemia (ALL). It has been associated with purine nucleoside analogues (PNA), but potential relationship with asparaginase has also been described. Despite these reports, clinicians’ awareness of this risk seems to be limited.

Results: In total, 28 hypoglycemic events were reported in 26 of the 3293 patients. 25 events in 23 patients were described as symptomatic, to which further analysis was restricted (22 precursor B- and one T-ALL; 8 standard-risk, 12 medium-risk, and 3 high-risk). Age of patients ranged between 1.7 and 15.5 years at occurrence of symptomatic hypoglycemia. Balanced ratio between both sexes can be observed (13 male, 10 female), median age was 3.9 years for both sexes, and 4.1 years for females.

Summary/Conclusions: Comprehensive IGH Assays have been developed for both MiSeq and PGM platforms. These assays identify clonal IGH V-J rearrangements and provide the clonal DNA sequences of the tumor-specific clontypes required to perform follow up testing to detect residual disease. Combining FR1, FR2 and FR3 improved the overall clonality detection rate to 96%. Both NGS-based IGH assays have demonstrated excellent concordance in detecting clonality regardless of whether clonality was determined using a PCR-CE method or with assays formatted for the MiSeq and PGM platforms.

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 IGL/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.

E848

HYPOGLYCEMIC EVENTS DURING TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: OBSERVATIONS FROM TRIAL AIEOP-BFM ALL 2009

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Aims: Descriptive evaluation of symptomatic hypoglycemic events during ALL treatment.

Methods: Hypoglycemic events were analyzed among 3293 patients treated in the trial AIEOP-BFM ALL 2009 in four of the participating countries (Germany, Switzerland, Czech Republic, and Australia) between 06/2010 and 08/2016. PNA were administered during induction-consolidation, the second part of the reintensification phase (reinduction-consolidation) and during maintenance (MT). Pegylated asparaginase (PEG-ASP) was given in induction and during reinduction, as well as high-risk blocks. Additionally, the benefit of intensified PEG-ASP was tested during induction-consolidation in the high-risk group, and during reinduction-consolidation/MT in the medium-risk group. Adverse events were generally captured in a targeted approach by means of defined events assessed as clinically relevant, not including hypoglycemia. Thus, data collection of these events was based on proactive reporting by the investigators. For analysis, clinical severity of the events was retrospectively graded according to patients' capacity of action and reaction.

Results: In total, 28 hypoglycemic events were reported in 26 of the 3293 patients. 25 events in 23 patients were described as symptomatic, to which further analysis was restricted (22 precursor B- and one T-ALL; 8 standard-risk, 12 medium-risk, and 3 high-risk). Age of patients ranged between 1.7 and 15.5 years at occurrence of symptomatic hypoglycemia. Balanced ratio between both sexes can be observed (13 male, 10 female), median age was essentially similar (male 3.2 y, female 4.1 y). Hypoglycemic events occurred in induction treatment (n=1), induction-consolidation (n=8), reinduction-consolidation (n=4; one in standard MT, 3 in reinduction with intensified PEG-ASP treatment), high-risk block (n=1), and in MT (n=11; 4 events during standard MT, 6 events during MT with intensified PEG-ASP treatment, and one event 4 weeks after last PEG-ASP during MT). Seven events were reported with mild symptoms, 6 patients showed moderate symptoms, and in 12 events patients showed severe symptoms (loss of consciousness, seizure-like).

Summary/Conclusions: In accordance with previous reports, hypoglycemic events accumulated in PNA containing treatment phases, but not exclusively. Considering that 324 patients of the total cohort were treated with intensified PEG-ASP in reinduction-consolidation/MT, an additive effect of PEG-ASP and PEGase could be assessed. A hypoglycemic metabolic condition may be assumed although a similar effect was not seen in induction-consolidation with intensified PEG-ASP. However, numbers are small and reporting bias of the present data is probable, as hypoglycemic events were not captured systematically. Investigators' attention to adverse reactions and proactive reporting might be higher.
in experimental arms as well as in case of preceding hypoglycemic events in
other patients of the respective trial center. Despite these analytical limitations,
our data suggest that hypoglycemia during ALL treatment is a relevant and
probably underestimated clinical problem. Further investigation including pos-
sible identification of predisposing metabolic conditions is required to avoid
harm to patients by this preventable complication.

E850
NUDT15 VARIANT IN KOREAN CHILDREN WITH ACUTE LYMPHOBlastic LEUKEMIA
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College of Medicine, Daegu, Korea, Republic Of

Background: Acute lymphoblastic leukemia (ALL) is the most prevalent pedi-
atric cancer with cure rates approaching 90% with current therapy. Patient with
ALL require long-term maintenance therapy. The combination of weekly methotrexate and daily 6-mercaptopurine (6-MP) is used as the backbone of
ALL maintenance regimens. Genetic polymorphism in thiopurine methyl-
transferase (TPMT) is well known to affect the 6-MP tolerance. However preva-
lence of non-function variant of TPMT is rare in Far East. Recently, a study has
identified a variant of the NUDT15 gene associated with intolerance of 6-MP.

Aims: We examined the association between NUDT15 polymorphism and clinical
data of Korean pediatric ALL.

Methods: NUDT15 genotyping and collection of clinical data was performed
for 74 Korean pediatric ALL patients from two different hospital. For NUDT15
 genotyping, DNA was extracted from whole blood/or bone marrow sample and
Sanger sequencing was performed for exon 1 and 3 of NUDT15 gene. 6-MP
dose intensity, defined as the ratio of prescribed 6-MP dose over protocol
planned dose.

Results: We found two kinds of variants, c.55_65insGAGTGC(rs686920766)
in exon 1 from 8 patients and c.415C>T(rs116855232) in exon 3 from 14
patients. Of them, 7 patients had both variants and all variants were heterozy-
gotes. Patients could be divided to four distinct groups according to combina-
tions 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</th>
<th>Relapse</th>
<th>Admission days during maintenance (Mean)</th>
<th>Sypera EFS (%)</th>
<th>Sypera OS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6M9 (C/T)</td>
<td>26</td>
<td>132.5</td>
<td>154.2</td>
<td>98.6 ± 2.5</td>
<td>98.2 ± 1.8</td>
</tr>
<tr>
<td>w.415C&gt;T</td>
<td>31</td>
<td>132.9</td>
<td>104.7</td>
<td>95.7 ± 12.7</td>
<td>90.0</td>
</tr>
<tr>
<td>c.415 C&gt;T</td>
<td>3</td>
<td>107.5</td>
<td>78.3</td>
<td>11.7</td>
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<tr>
<td>c.415 C&gt;T + c.55_65insT</td>
<td>2</td>
<td>65.0</td>
<td>85.0</td>
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<tr>
<td>c.415 C&gt;T + c.55_65insC</td>
<td>3</td>
<td>107.5</td>
<td>104.7</td>
<td>95.7 ± 12.7</td>
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<tr>
<td>C.55_65insGAGTGC (C/T)</td>
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<td>88.3</td>
<td>64.8</td>
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</tr>
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</table>

Figure 1.

Summary/Conclusions: Genotyping of NUDT15 could be beneficial to predict
the tolerable dose of 6-MP of pediatric ALL patients.

E851
Abstract withdrawn.

E852
TREATMENT OUTCOME OF ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREAN ADOLESCENTS AND YOUNG ADULTS
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Background: The outcome of acute lymphoblastic leukemia (ALL) has marked-
ly improved for last centuries, but the improvement was mainly observed in children under 10 years old. In contrast, the treatment outcomes of ALL in ado-
lescents 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 out-
come of AYA ALL.

Methods: Clinical data of 10-29 years old ALL patients diagnosed between
2002 and 2010 were extracted from Korean national health insurance service.

Data about patients’ diagnosis, age, gender, mainly treated department (internal
medicine vs pediatrics), usage data of medications (L-asparaginase, 6-mer-
captopurine, vincristine, prednisolone or dexamethasone), hematopoietic stem cell
transplantation (HSCT), radiotherapy, survival, and follow-up duration were
collected. Patients who were treated with steroid over 2 weeks, and L-aspar-
gagine at least once in initial 2 months were considered to be treated as pediatric
protocol, and who did not fulfil this criteria were considered to be treated as
adult protocol.

Results: Total 1,223 ALL AYA patients were diagnosed between the 2002 and
2010, and excluding those who never treated, 1,208 patients underwent ALL
treatment. Among them, 665 (55%) patients were treated with pediatric protocol,
and the other 543 (45%) patients were treated with adult protocol. Radiotherapy
was done in 278 (41.8%) and 186 (34.3%) in each group, and HSCT was done
in 205 patients (30.8%) and 216 patients (39.8%) in each group, respectively.

Pediatric protocol group showed significantly better overall survival compared
to adult protocol group in total age (65% vs 43%, P<0.0001), 10-14 year old
(76% vs 57%, P<0.0001), and 20-24 year old patients (51% vs 31%, P=0.0116).

In univariable analysis, patient age (younger), treatment protocol (pediatric), L-
Asparaginase, 6-mercaptopurine, and steroid over 2weeks in initial 2 months
were associated with better overall survival (P<0.0001 for each).

Summary/Conclusions: The overall survival rates in Korean AYA ALL were
comparable with previous studies done at other countries. Patients treated with
pediatric protocol tended to result better overall survival rate when compared
to patients treated with adult protocol. Radiotherapy and early HSCT were wide-
ly used in the 2000s, and further study is needed to follow up the recent trend
of treatment, and outcome as a result.

E853
AUTOLOGOUS TRANSPLANTATION AS TIME-DEPENDENT FACTOR FOR SURVIVAL OF PATIENTS WITH T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA: STUDY DATA AND SIMULATION MODEL
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Background: The role of autologous hematopoietic stem cells transplantation
(aHSCT) for patients with T-cell ALL is still being discussed. The recent Russia
study of ALL shows the promising effect of aHSCT but there is a skepticism as
the study was not randomized. The possible bias was referred to the “time selec-
tion” factor.

Aims: It’s need to prove that time selection can not explain the magnitude of
the effect of aHSCT on patient’s survival.

Methods: We have developed SAS macros time-depend graphical and analytic
procedures for time dependent factors: Land Mark (LM) methods, Mantel-Bay-
test, Cox regression model (CM) and also a base for simulation all end points
and study events like remission, transplantation, relapse and death are well
assessed by a mixture of exponent distributions. Non-constant (time varying)
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 sim-
ulation model in different situations. Real data multicenter study of ALL was
used to fit simulation model parameters. Russian ALL study group held a prospec-
tive multicenter trial RALL-2009 in the treatment of Ph-negative adult
ALL patients based on non-intensive but non-irruptive treatment
(NCT01193933). The therapy was unified for all Ph-negative ALL pts, but in T-
cell ALL/LBL autologous hematopoietic stem cell transplantation (auto-HSCT)
after non-myeloablative BEAM conditioning was scheduled as late intensifica-
tion (+3-4 mo of CR) followed by prolonged 2 years maintenance. From Jan
2009 till Jul 2016, 30 centers enrolled 107 T-ALL/LBL pts. Median age was 28
years (15-54 y), 34 f / 73 m; early T-cell (T/II) phenotype was verified in 56

Madrid, Spain, June 22 – 25, 2017
(52.3%), mature (T-IV) - in 10 (9.4%), thymic (TII, CD1a+) ALL - in 41 pts (38.3%). T-lymphoblastic lymphoma (T-IVL: <25% b/m blasts) was diagnosed in 22 pts (20.5%). Autologous HSCT was performed in 35, allogeneic-in 7 pts.

**Results:** The survival analysis of real data shows 4-fold dropping hazard rate. The effect of aHSCT was confirmed by LM analysis, Mantel-Bay test - P-value=.0004, Cox model output: 1/HR=15.9, P=0.008. (Fig.1). Simulation model for remission consists of 3 fractions: early (α=10%, τ=0.05 m, δ=0.2 m), normal (α=57%, τ=0.28 m, δ=1 m) and late remission (α=33%, τ=1.31 m, δ=2.2 m), for survival consists of 2 fractions: short life (α=59%, τ=22 m), long life (α=41%, τ=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<0.05). The second experiment supposed that the existed effect of aHSCT (HR=0.5), N=500. Mantel-Bay and Cox model would show significance, but hazard ratio was underestimated (P<0.03, P<0.05, HR=0.70 (0.50-0.97)). More experiments were done for repeated simulation, which demonstrated a very good agreement of Mantel-Bay and Cox methods and their robustness.
JL1 is a novel epitope of CD43, which is known to be specifically expressed in leukemic cells. The antigen is not expressed on mature peripheral blood hematopoietic cells. The antigen is expressed depending on the differentiation stages of hematopoietic cells. JL1 was examined using FACSCanto II (Becton-Dickinson, Sunnyvale, CA).

Methods: Pts were recruited if aged ≥ 18 years; with de novo Ph+ ALL or CML-LyBP treated by PON alone or in combination for at least 1 treatment day, for relapsed or refractory disease, between Apr 2012 and Dec 2014 (Expanded LyBP, treated by PON alone or in combination for at least 1 treatment day, for gone hematopoietic stem cell transplantation (HSCT). Of the 18 pts screened for BCR-ABL mutations, 5 had none, 3 had T315I, 3 had other PON-sensitive mutations, while 5 had compound mutations (known to be resistant to all TKIs including PON) and 2 had E255V (of intermediate sensitivity to PON). PON was administered alone in 13 pts, combined to low-intensity chemotherapy in 6 pts, intensive chemotherapy or monoclonal antibody in 2 pts. Dose at initiation was 45mg in 17 pts and 30mg in 4 pts.

Results: Median duration of PON therapy was 3 months (5 days-30 months+). Out of the 19 pts who received PON for ≥ 4 weeks, 5 pts failed to reach CR, while 14 (78%) reached or maintained it. Molecular response was not reported uniformly. During induction by PON, 5 pts stopped therapy in 4 pts (1 pulmonary infection; 1 acute renal failure; 1 pancreatitis; 1 hepatitis; 1 venous thrombo-embolic event; no arterial occlusive event). Post-induction therapy consisted in PON-based therapy in most pts. HSCT was performed in 5 Pts. Our study shows that 1 pt died in CR on PON, 1 pt died in CR from acute stem cell failure in the HSCT process at 8 months, and 11 pts ultimately experienced bone marrow relapse, all of them within 6 months after PON initiation, except 2 who relapsed at 13 and 27 months after HSCT. Two patients are alive in CR at 14 and 30 months, 1 pt is still on PON.

Summary/Conclusions: Our series of resistant pts is comparable to the PACE study population by initial characteristics and high frequency of BCR-ABL mutations. CR was achieved in most pts, suggesting the role of PON as a bridge to HSCT. Because global outcome of this very high-risk population remains poor, earlier introduction of PON in the course of the disease is warranted, as underlined by the excellent results of the hyperC-VAD-PON combination in the first-line setting.

E857

JL1 ANTIGEN EXPRESSION OF LEUKEMIC CELLS IN CHILDHOOD ACUTE LEUKEMIA

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Background: JL1 is a novel epitope of CD43, which is known to be specifically expressed depending on the differentiation stages of hematopoietic cells. JL1 antigen is expressed on tumor cells of T, B, and myeloid lineage in >80% of acute leukemia patients, and its expression is limited in normal multipotent hematopoietic cells. The antigen is not expressed on mature peripheral blood cells or other normal tissues. Thus, the clinical phase 1 test of a therapeutic agent for leukemia targeting JL1 is being conducted, and when anti-JL1 antibody was combined with a toxic substance, its therapeutic effect was found earlier in preclinical trials.

Aims: This study aims to examine JL1 expression of leukemic cells in childhood acute leukemia.

Methods: Between December 2014 and January 2016, a total of 71 patients younger than 21 years with acute myeloid leukemia (AML, n=25), and acute lymphoblastic leukemia (ALL, n=46) were enrolled in this study. Expression of JL1 was examined using FACSCanto II (BD Biosciences, 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 staining did not show significant difference between B-lineage 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 demonstrates 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 LYMPHOPROLIFERATIVE LEUKEMIA PATIENTS

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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, cytokine receptors and adhesion molecules in newly diagnosed acute lymphoblastic leukemia (ALL) patients and to assess their correlation with baseline characteristics and prognostic factors.

Methods: A total of 30 newly diagnosed ALL patients (median age 46, range 22–75 years, 20 males) were included. We evaluated serum levels of 31 analytes, specifically 21 cytokines, 4 soluble cytokine receptors, 5 soluble adhesion molecules and Matrix Metalloproteinase-9. From cytokines, we measured Interleukins (IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-23), Epidermal Growth Factor (EGF), Granulocyte Macrophage Colony Stimulating Factor (GM-CSF), Interferon-γ (IFN-γ), Macrophage Inflammatory Protein-1α (MIP-1α), Monocyte Chemotactic Protein-1 (MCP-1), Tumour Necrosis Factor-α (TNF-α), Vascular Cell Adhesion Molecule-1 (VCAM-1) and E-selectin (E-selectin), P-selectin (P-selectin), Intracellular Adhesion Molecule-1 (ICAM-1) and Vascular Cell Adhesion Molecule-1 (VCAM-1). All analytes were measured by biochip array technology on Evidence investigator analyzer (Randox). Serum levels of tested analytes were correlated with baseline characteristics using software R 3.3.2 (R Core Team 2016).

Results: Comparing analytes with baseline characteristics, we found significant negative correlations between IL-7 and leucocyte 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 lymphocyte count (r=0.801; p=0.001), VCAM-1 and LDH (r=0.664; p=0.012). Correlations with baseline risk stratification according to GAML all did not reach statistical significance. In the study population, CR rate after induction was 86% (MRD negative in 29%), 1-year PFS 68% and 1-year OS 73% (2 patients died during induction therapy). Higher levels of EGF were associated with failure to achieve CR after induction therapy (r=0.696; p=0.073). 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=0.001), IL-1α and IL-4 (r=0.700; p=0.008), sTNFR-2 and MIP-1α (r=0.657; p=0.037), sTNFR-2 and VCAM-1 (r=0.652; p=0.044). Correlations between adhesion molecules did not reach statistical significance.

Summary/Conclusions: Our findings show that serum levels of IL-7 and VCAM-1 are associated with some baseline characteristics of newly diagnosed ALL patients and EGF with response to induction therapy. Better understanding of leukemia microenvironment is essential for development of new treatment approaches. Further studies in this field are warranted.

The work was supported by a long-term organization development plan 1011 (FMHS) and by MH CZ – DRO (UHKH, 00719906).
IMATINIB VS. DASATINIB FOR OUTCOMES AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION IN PATIENTS WITH PH+ ACUTE LYMPHOBластIC LEUKEMIA

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Background: The survival of the patients with Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL) who received allogeneic stem cell transplant (allo-HSCT) has improved over the development of tyrosine kinase inhibitors (TKIs). Currently, Imatinib (IMA) and Dasatinib (DAS) are widely used for the treatment for Ph+ALL. However, there has been no data comparing the outcomes between the patients who received allo-HSCT and the two distinctive TKIs respectively.

Aims: We conducted a retrospective analysis for comparing the two TKIs for the outcome after allo-HSCT.

Methods: Clinical data of patients were retrospectively collected from Hokkaido University Hospital and Sapporo Hokuyu Hospital. The patients’ eligibility were as follows: diagnosis 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 treatment 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. Other characteristics, such as age, disease status at diagnosis, stem cell source were not significantly different between the two groups. Incidences of Neutrophil engraftment and acute GVHD incidence were not statistically different between IMA-pts and DAS-pts. Incidence of chronic GVHD was marginally increased in IMA-pts (IMA; 63%, DAS; 33%, P=0.08). At the median follow-up of 34 months (range: 14-462 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-HCT 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 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; NCT01193933) with concurrent administration of Imatinib. This protocol includes 8 cytostatic drugs and no intervals between treatment phases. Since 2012 till now 23 pts were included in ongoing RALL–2012 protocol, based mainly on 600 mg Imatinib with prednisolone, VNCR, L-asp, followed by 6-MP and MTX. Both protocols suggested the shift to Dasatinib (100-140mg) after non-achievement of MolCR on day 70 of treatment. MolCR was stated if bcr/abl chimeric transcript was <0.01% by PCR with 10-4 sensitivity. All patients were considered as candidates for allogeneic HSCT if HLA-identical donor was available. 13 pts (37%) underwent allo-HSCT as the first-line therapy: 1 autologous, 5 matched related and 7 matched unrelated.

Results: Six RALL–2009 patients were included in ongoing RALL–2012 protocol and 2 cases on RALL–2009. Hematological CR was achieved in 30 (85.7%) of 35 pts (except four early deaths and 2 refractory cases). There was one autologous HSCT in MolCR on the first protocol. Allo–HSCT was carried out in 5 of RALL–2009 protocol pts and in 9 of RALL–2012. The major issue was the non-relapsed mortality after unrelated allo–HSCT in 3 older pts (49, 56 and 59 years old) who were included in RALL–2012 (aGVHD and severe infections, at a median +4 months after HSCT and more than 12 months of CR duration). The 5y overall survival (OS) and relapse-free survival (RFS) for all 35 pts constituted 54.6% and 40.4% respectively. The long-term outcome on both protocols and at different ages are presented in Table 1. OS6 years in transplanted group of the pts, and RFS was 55.7% vs 45% (p=0.7), respectively. In order to evaluate the impact of allogeneic HSCT we performed a comparison of transplanted and non-transplanted patients by a landmark analysis. The landmark was chosen as the median time from CR to allo–HSCT – 4,3 mo (3-16 mo). So, the 5y OS from landmark was 53.3% for non-transplanted patients and from day of HSCT – 65.6% in transplanted (p=0.18), and RFS was 25% vs 62.5% (p=0.19), respectively. OS for older pts (>45 y) was 40% vs 25% in transplanted vs non-transplanted group of the pts, and RFS was 25% vs 66.6%, respectively. OS in younger (≤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 BLINatumOBAmb + 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.

Results: During this period, a total number of 353 patients with childhood ALL were treated in our Department, according to BFM protocols. Recurrence occurred in 86 patients (24.4%, 56 male - 30 female - median age: 4.83 years), within 3 to 184 months from initial diagnosis. Very very late recurrence was noted in 3.1% of our relapses (8 male - 3 female) at 53, 72, 82, 83, 84, 108, 112, 116, 120 and 184 months from initial diagnosis. In 9 patients recurrence immunophenotypically was 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 hypodiploidy, one del(6)(q12), one BCR-ABL fusion and one 47.XY,i(19)(q13); 2 del(12)(p13); 3 other rearrangements. In ALL disease, the cytogenetic profile remained identical at recurrence, while in 1, trisomia 13 was not detected and another had heterozygous absence of IKZF1, PAIX5, EBF1, CDKN2A and CDKN2B genes. On Day 8, none of 11 patients were Prednisone Good Responders. On Day 15, nine children had bone marrow M1, one M2 and one M3, and on Day 33 only one had M2. Two patients were classified as low risk, 6 as intermediate risk and 3 as high risk. Second remission (CR2) was achieved in 9 children with very very late recurrence. The other 2 died from disease progression. Six of nine patients are still alive and well 6, 8, 10, 10, 11 and 20 years after initial diagnosis. One patient died from second recurrence and the last two had a second allogeneic BMT and died due to severe infection, 2 and 12 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 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.

E683

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: This study is 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 T- and B-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 CRLF2 NO Immunogenetic Diagnosis and Classification of ALL (2008). Mutational analysis of CRLF2 exons 1-6 was performed. Genomic DNA was isolated and DNA fragments spanning the aforementioned CRLF2 exons were amplified by PCR using AmpliTaq Gold kit (Applied Biosystems, USA) and the exon-specific primers. The resulting PCR products were purified by EXOSAP-IT (USB, USA) and sequenced by the Beijing Genomics Institute (BGI; Beijing, China). The frequency, positions, types and clinical significance of CRLF2 mutations were analyzed. For qualitative parameters, overall group differences were analyzed using a χ2 test. All statistical analyses were performed using SPSS version 17.0 (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate statistical significance.

Results: Six novel CRLF2 mutations were detected in the 129 patients without CRLF2 rearrangement, which were L86I (0.8%), R186S (7.8%), P224L (8.5%), W255C (0.8%), and two silent mutations F232F (0.8%) and A11A (12.4%). The overall rate of CRLF2 mutation was 26.6%. Exon1 contained three types of mutations. The incidence of A11A in B-ALL was significantly higher than in T-ALL (14.7% vs 2.4%, P=0.037), whereas R186S was only detected in B-ALL. Exon1, exon5 and exon6 mutations were detected in B- and/or T-ALL patients; but no mutations were detected in exon2 and exon4. None of these mutations were reported in the COSMIC and SNP databases. The patients with R186S, P224L mutations showed significant differences with that of non-mutant patients in sex, age, white blood cell count, hemoglobin level, and platelet count. The median neutrophil count in the patients with P224L mutation
was lower than that of non-mutation (8.5×10⁹/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 mutation (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 with the first induction chemotherapy. Interestingly, the patient with silent mutation, A11A showed higher age (46 vs 30 years, P=0.033), higher HLA-DR (100% vs 75.3% P=0.035), CD22 (93.3% vs 47.4% P=0.020) than those without the mutation; and the patient with F232F mutation relapsed in 6 months.

Summary/Conclusions: Six novel CRLF2 genetic mutations were identified in adult ALL patients and may associate with clinical outcome, such as CRLF2 R186S indicating favorable, while L86I and W255C indicating poor outcome. Our data indicated that the CRLF2 mutations may be new prognostic markers and play an important role on oncogenesis in ALL.

Acute myeloid leukemia - Biology

E864

THE MUTATIONAL SPECTRUM OF T(8;21)(Q22;Q22) POSITIVE ACUTE MYELOID LEUKEMIA DETERMINED BY HIGH-THROUGHPUT TARGETED SEQUENCING

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Background: Recently, comprehensive genetic profiling of pediatric and adult core-binding factor (CBF) AML revealed a variety of cooperating events in a cohort of 85 t(8;21) AML patients (Faber et al. Nat Genet 2016). These mutations comprised alterations in genes encoding for proteins in tyrosine kinase (TK) signaling, epigenetic regulation (ER), and in the cohesin complex (CC).

Aims: To validate and to further extend our recent findings by comprehensive characterization of the mutational landscape of t(8;21) positive AML using a high-throughput targeted sequencing (HTS) approach.

Methods: The HTS panel comprised the entire coding region of 244 genes that are involved in hematological malignancies. Pretreatment blood (n=23) or bone marrow specimens (n=72) of 95 additional adult t(8;21) positive AML patients (pts) (median age: 51 yrs, range 18-72 yrs) were analyzed. 92/95 pts were enrolled in one of seven prospective AMLSG treatment trials. Libraries (total probe size: 1.359 Mbp) were prepared using SureSelectXT custom solutions (Agilent). Paired-end sequencing was carried out on a HiSeq 2000 (Illumina). The variant allele frequency (VAF) cutoff for reporting mutations was set at ≥0.05.

Results: The median coverage per pt was 900x. Mutations were detected with an average of 5.1 (SD: ±2.6) per pt with 99% of all pts harboring at least 1 mutation and 87% ≥ 3 mutations. Consistent with previous studies, mutations in TK signaling pathways were common events: KIT mutations were found in 22/95 pts (23%) followed by mutations affecting NRAS (16/95; 17%), FLT3 (11/95; 12%; point mutations only), and KRA5 (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 DDX15 (6/95; 6%) being involved in spliceosome function and ribosome biogenesis. With respect to the clonal architecture we found that the median VAF in genes belonging to ER and CC (0.30; range 0.03-0.91; 0.31, range 0.05-0.73, respectively) was higher than in genes associated with TK signaling (0.19, range 0.05-0.53). These data suggest that alterations affecting the epigenetic state and differentiation occur earlier than those in signaling during t(8;21) leukemogenesis.

Summary/Conclusions: Using a comprehensive, deep sequencing approach we could further characterize the mutational landscape of t(8;21) positive AML. Here, mutation clusters in genes involved in TK signaling, ER and CC were confirmed as well as novel CBF-associated gene mutations that play an essential role in regulation of hematopoietic cell proliferation and differentiation. Further analyses in terms of sample size extension as well as correlation of findings with clinical parameters are ongoing.

E865

NFkB PATHWAY PROMOTES TUMOR PROGRESSION THROUGH BRUTON'S TYROSINE KINASE IN MLL+ ACUTE MYELOID LEUKEMIA

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A PRECISION MEDICINE PLATFORM FOR ACUTE MYELOID LEUKEMIA

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. 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) (Pemovska 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 for AML. We are also interested in uncovering possible downstream target/s of BTK, improving the therapeutic efficacy of the drugs used.

Results: 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 5µM) or RAC (NSC 23766; NSC: 5, 10, and 20µM) for 48 hrs and cell viability was assessed using Annexin V/Sytox Blue based flow cytometric analysis.

Conclusions: These findings present a novel treatment that has remained unchanged for the past 30 years (Longo et al., 2013). Current standard of care for AML is an aggressive cytostatic treatment that has high drug dose-related side effects but increasing the therapeutic efficacy.

AIDS: CD3/CD23 BITEN® ANTIBODY MEDIATED CYTOTOXICITY AND T-CELL PROLIFERATION

Aims: In our previous work, we showed that the CD3/CD23 BITEN® antibody construct (AMG 330) is able to recruit autologous, residual T-cells and induce cytotoxicity against primary AML cells ex vivo. However, as described previously (Mussai 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.

Methods: We used plasma samples (from heparanized serum tubes or after density gradient centrifugation) from newly diagnosed and relapsed AML patients in AMG 330 cocultures of healthy donor (HD) T-cells and AML cell lines. In flow cytometry based experiments we determined the influence of AML plasma in comparison to fetal calf serum (FCS, heat inactivated) on AMG 330 mediated cytotoxicity and T-cell proliferation. In transwell experiments using primary AML cells physically separated from AMG 330 cocultures, we evaluated if AML cells are the source of soluble factor secretion.

Results: The influence of AML plasma from bone marrow (BM) of AML patients on AMG 330 mediated cytotoxicity and T-cell proliferation was heterogeneous: In 15/30 samples, AMG 330 mediated cytotoxicity was significantly reduced compared to cultures containing FCS (mean% specific lysis FCS vs BM: 72.8 vs 26.8). This was accompanied by a reduction in T-cell proliferation (mean% proliferation FCS vs BM: 27.7% vs 9.5%). The degree of immunosuppression could not be correlated to percentage of bone marrow blasts. Interestingly, the effect was not observed in AML plasma samples from peripheral blood (PB) (mean% specific lysis FCS vs PB: 84.7 vs 83.5%; proliferation FCS vs PB 32.2 vs 26.5%, n=15). In the remaining 15 samples from AML BM no influence on AMG 330 mediated T-cell function was observed (mean% specific lysis FCS vs BM: 82.1 vs 78.3%; proliferation FCS vs BM: 25.7 vs 26.8). In control cultures plasma from AML patients in complete remission (CR) or from HD BM was used which did not negatively impact AMG 330 mediated cytotoxicity (mean% specific lysis FCS vs CR: 76.6 vs 78.3%; proliferation FCS vs CR 3.2 vs 5% FCS vs HD; 36.6, n=6) and 56 AML samples from AML 78.6 vs
41.8, n=7). In the remaining 7 primary AML samples, no immunosuppressive effect was observed specific for tyrosine kinase control vs AML 98.9 vs 98.2% proliferation control vs AML 82.8 vs 77.7, n=7).

Summary/Conclusions: In summary we demonstrated that BM derived plasm-a from AML patients at primary diagnosis or relapse reduced T-cell proliferation and AMG 330 mediated cytotoxicity in half of the samples tested. The immunosuppressive effect was observed in primary AML cell samples treated by transwell experiments. Unraveling mechanisms of resistance to BiTE® antibody mediated cytotoxicity will allow the exploitation and usage of enhanced strategies to increase response rates.

E868

CLONAL EVOLUTION OF FLT3-ITD POSITIVE AML AT DIAGNOSIS AND RELAPSE IN PATIENTS TREATED WITHIN THE CALGB 10603 (RATIFY) AND AMLSG 16-10 TRIALS

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Background: Internal tandem duplications (ITD) in the receptor tyrosine kinase FLT3 occur in about 22% of patients (pts) with acute myeloid leukemia (AML) and confer a poor prognosis depending on the mutational load. The multi-targeted kinase inhibitor (TKI) midostaurin has been shown to improve outcome in FLT3-ITD positive (FLT3-ITDpos) and FLT3-TKDmut pts in combination with intensive chemotherapy [CALGB 10603 (RATIFY) trial], thus representing a promising targeted treatment approach. However, a significant number of pts relapse after initial response due to yet unknown mechanisms.

Aims: To study the clonal evolution in FLT3-ITDpos pts treated in the AMLSG 16-10 (NCT01477606) or CALGB 10603 (RATIFY, NCT00651261) trial in paired samples obtained at diagnosis (Dx), complete remission (CR) and relapse (Rel) by whole exome sequencing (WES).Methods: WES was performed in 17 FLT3-ITDpos pts using the Nextera Rapid Capture Exome kit (Illumina) for library preparation followed by sequencing on a Illumina HiSeq2000. 6 pts were treated in the RATIFY trial receiving either midostaurin or placebo combined with intensive chemotherapy during induction and consolidation and 11 pts treated in the CALGB 16-10 trial. In the CALGB 16-10 trial treatment with midostaurin combined with intensive chemotherapy during induction and consolidation followed by a one-year maintenance therapy with midostaurin; 4 pts in the AMLSG16-10 trial received allogeneic hematopoietic cell transplantation. The presence of FLT3 and NPM1 mutations (mut) and the allelic ratio (AR) of FLT3-ITD were analyzed according to standard protocols.

Results: The median AR of FLT3-ITD was 0.51 (0.10-18.94) and 0.54 (0.07-26.31) at Dx and Rel, respectively. Loss of FLT3-ITD was observed in 5 pts; changes of the ITD clone at Rel occurred in 7 pts. Of those, 5 pts had a change of FLT3-ITD that was lost at Rel. 6 pts had a NPM1mut that persisted at Rel in all pts. Using WES, 301 mut (226 missense, 24 nonsense, 41 indels, 6 splice sites, 4 unique ideplets) were identified. The average coverage was 125 (186-67) among all samples. 131 (43%) mut were present at both time points (Dx and Rel), 149 (53%) mut were only subclonal at Dx (RE5 73 (24%) mut were detected only at Rel and 14 mut with only 1 read at Dx. Besides FLT3-ITD, the average number of mut per sample (Dx or Rel) was 13. Mut were most frequently observed in genes related to signaling (23%), transcription (20%), DNA methylation (5%), chromatin biology (4%), components of the mitotic machinery (4%), Pre-leukemic (DNMT3a, TET2, IDH1/2) were detectable in 10 pts at both time points and persisted at CR in 7 pts. Mut resistant in transcription mutations occurred genes in 8 pts at Dx and Rel, with WT1 mut being most frequent (n=7). Mut in signaling related genes present at both time points included NRAS (G12V/D) and NFI mut. At the time of Rel, gene mut frequently referred to signaling (34%) including a KRAS (G13D) and a KIT (D816V) mut, both in pts with loss of FLT3-ITD at Rel.

Summary/Conclusions: Analyzing the clonal evolution of FLT3-ITDpos AML, known pre-leukemic mut were stably detectable at Dx and Rel in most pts, whereas unknown unexpected gene mutations were detected only at Rel. We investigate more comprehensively pathways underlying therapy resistance with a focus on TKI treatment, larger cohorts of pts are currently analyzed for the detection of recurrent mutational patterns.
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Background: Internal tandem duplications (ITD) and mutations (mut) in the tyrosine kinase domain (TKD) of the receptor tyrosine kinase FLT3 occur in about 25% of acute myeloid leukemia (AML) patients (pts). FLT3-ITD is associated with an unfavorable prognosis in particular in pts with a high allelic mutant to wildtype ratio (AR>0.5) as well as localization of the ITD in the beta1-sheet of the receptor. FLT3is targetable by tyrosine kinase inhibitors (TKI) and the combination of chemotherapy with the TKI midostaurin has been recently investigated within the CALGB 10603 RATICY trial and is still under investigation within the AMLSG 16-10 trial.

Aims: To study the FLT3mut status at the time of diagnosis (Dx), refractory disease (RD) and relapse (Rel) in AML pts treated within the CALGB 10603 RATICY trial and AMLSG 16-10 (NCT01477606) trial with regard to AR of FLT3-ITD and FLT3-TKDMut, loss of FLT3-ITD and FLT3-TKDmut and change of ITD clones (ITD insertion site, length, number of clones).

Methods: FLT3-ITD and FLT3-TKDMut were detected using Genescan-based fragment-length analysis according to standard protocols. In the randomized phase-Ill RATICY study, FLT3mut pts were treated with induction (daunorubicin/cytarabine) and consolidation (high-dose cytarabine) and maintenance therapy in FLT3-ITD positive pts.

Results: In total, 83 pts were analyzed, of which 33 were treated in the RATICY and 50 within the AMLSG 16-10 trial. 36 pts had RD and 47 pts had relapsed. FLT3-ITD was present at diagnosis in all pts treated in the AMLSG 16-10 trial; one pt had an additional FLT3-TKDMut. Pts entering the trial had either a FLT3-ITD (n=22), a FLT3-TKDMut (n=9), or both (n=2). The median AR of FLT3-TKDMut at Dx was 0.82 (0.07-2.66) and the majority of pts showed loss of FLT3-TKDMut at RD or Rel (n=9/12; 75%). In relapsed pts, loss of FLT3-ITD occurred in 14 (36%) pts. There was no significant difference between the median FLT3-ITD AR at Dx [0.02 (0.10-14.94)] and Rel [0.05 (0.07-38.75); p=0.98]. A switch of the ITD clones was found in 14 (36%) pts at Rel, with switch of the ITD insertion site or length in 8 (21%) pts. 1/8 pts with change of the ITD clone at Rel had multiple ITD clones at Dx. For 35 FLT3-ITDPositive pts with refractory AML, FLT3-ITD loss was observed in 17 (49%) pts. The median AR of FLT3-ITD was significantly lower at the time of RD [0.29 (0.05-2.37)] compared to Dx [0.05 (0.08-9.11); p=0.02]. The ITD clone changed in 5 (14%) pts with RD. In pts with shift of the ITD clone at RD (n=5) or Rel (n=14), no significant difference of the median ITD length was observed (p=0.84).

Summary/Conclusions: Comparing the FLT3-ITD status at Dx, at the time of RD or Rel, we found a lower median AR of FLT3-ITD in pts at RD compared to Dx, whereas no significant change of AR was observed at Rel. In addition, loss of FLT3-ITD was observed in 49% of pts at RD and in 36% of pts at Rel. These findings suggest that the FLT3-ITD clone can be targeted in a significant number of pts and other clones might mediate resistance to treatment. We also observed a switch of the ITD clone in about 20% of pts with Rel, indicating the presence of ITD clones that might mediate failure of the available therapy. Despite the small number of TKD mutations in our study, it was remarkable that most of the TKDs (75%) were lost at the time of RD or Rel.

E872

COOPERATION OF MLL-PTD WITH DNMT3A OR RUNX1 MUTATIONS IN AML LEUKEMOGENESIS

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Background: Our previous study showed that DNMT3A or RUNX1 mutations were frequently coexisted in the MLL-PTD AML patients (Oncotarget 2015). We aimed to investigate the role of coexisted DNMT3A or RUNX1 mutations in leukemogenesis of MLL-PTD AML.

Methods: After lentiviral-mediated over-expression of RUNX1 or DNMT3A mutants in MLL-PTD mouse bone marrow (BM) cells or human MLL-PTD+ AML cell lines, colony formation, cell proliferation, differentiation and apoptosis assays were carried out. Interaction of RUNX1, HIF-1α, and MLL-PTD were evaluated by co-immunoprecipitation assay. Differential genes and protein expression, histone modifying protein expression, and enrichment of histone-4 acetylation (H4Ac) were assessed by RT-qPCR, Western blot, and ChIP-seq, respectively. For BM transplantation assays in mice, MLL-PTD+ BM cells over-expressing DNMT3A-wild type (WT) mutants and empty vector (EV) control were injected into C57BL/6 mice via tail vein.

Results: We observed that MLL-PTD mouse BM cells with RUNX1 mutants lacking C-terminal VWRPY sequence (H377fsX and V425fsX576) had increased self-renewal, proliferation, increased HIF-1α and its downstream gene expression, histone modifying protein expression, and enrichment of histone-4 acetylation (H4Ac) compared to EV or WT mutants at 10 days post-BM transplantation.

Summary/Conclusions: We present the study showed that both RUNX1 and DNMT3A mutants deregulated self-renewal, proliferation and apoptosis in the mouse MLL-PTD BM cells. Disruption of MLL-PTD-RUNX1-HIF-1α complex in the RUNX1-mutant and aberrant methylation in the DNMT3A-mutant cells might play an important role in AML pathogenesis. Our results showed that cooperation of RUNX1 or DNMT3A mutations had impact on leukemogenesis of MLL-PTD AML.

E873

AML BLASTS INDUCE A SENESCENT PHENOTYPE IN THE BM-MSC THROUGH THE UPREGULATION OF P21 ACTIVATING PROTEIN (P21) IN AML BLASTS INDUCE AN INHALATION

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Background: Acute myeloid leukaemia (AML) is a heterogeneous clonal disorder that arises from the haematopoietic myeloid progenitor cells within the
bone marrow microenvironment (BMM). Survival of patients with AML is presently poor; two-thirds of younger adults and 90% of older adults die of their disease. Even in patients who achieve remission with chemotherapy, relapse is common and occurs from minimal residual disease sequestered in protective niches in the BMM. Reciprocal interactions between that of the AML and bone marrow mesenchymal stromal cells (BM-MSC) are central to the survival and proliferation of blast cells through micro-environmental promotion of quiescence in malignant cells as well as the activation of anti-apoptotic and pro-survival pathways.

**Aims:** To investigate how BMM 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 (LRCEref07/H0310/146). Low input RNASeq of 10 AML BM-MSC and 10 healthy BM-MSC (taken from the pelvis of patients undergoing elective hip replacement surgery) was performed following CD271 MicroBead selection. Primary AML blasts and BM-MSC were 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 from the RNA sequencing carried out to compare 10 healthy and 10 AML BM-MSC show that 1125 genes were differentially expressed, with 924 down-regulated in AML derived BM-MSC and 201 up-regulated. From this analysis, we found that CDKN1A (p21) is up-regulated in BM-MSC from AML patients (7.406 logFC) compared to BM-MSC from patients with normal bone marrow (likely a marker for older adults). p21 mRNA expression was confirmed in AML BM-MSC compared to normal BM-MSC. In-vitro experimentation showed that p21 mRNA and protein expression is increased in BM-MSC when co-cultured with primary AML. Furthermore, we show that AML increased senescence β-Galactosidase staining in BM-MSC and that p21 knockdown in BM-MSC reversed this senescence phenotype. Finally, primary AML cultured on p21 knockdown BM-MSC had reduced survival compared to control BM-MSC.

**Summary/Conclusions:** We have identified that AML induces a senescent BM-MSC niche via the p21 mediated pathway which in turn promotes survival and proliferation of AML. Silencing of p21 within the BM-MSC reduces AML survival. In identifying this novel microenvironment feedback loop in AML we highlight a potential new target for future AML therapies.

**E875**

**BONE MARROW ECOLOGICAL COLLAPSE IN ACUTE MYELOID LEUKAEMIA IS MEDITATED BY REMODELING OF ENDOSTEAL VESSELS**

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**Background:** Bone marrow vascular niches have been proposed to support acute myeloid leukaemia (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 better characterize and study this heterogeneity, we developed an in vivo model of AML using patient derived xenografts (PDX). To better characterize and study this heterogeneity, we developed an in vivo model of AML using patient derived xenografts (PDX).

**Results:**

- Using a murine model of AML we performed intravital microscopy to better characterize and study this heterogeneity, we developed an in vivo model of AML using patient derived xenografts (PDX).

**Summary/Conclusions:** Together, these data suggest that AML-induced vascular damage contributes to cell egress from the bone marrow, and that new therapeutic approaches aiming to normalize bone marrow vasculature may support normal hematopoiesis.

**E876**

**CLONAL HETEROGENEITY IN PATIENT-DERIVED XENOGRAFT OF ADULT ACUTE MYELOID LEUKAEMIA**

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**Background:** Acute myeloid leukaemia (AML) is the most common leukaemia in adults. Currently, despite intensive chemotherapy and bone marrow transplantation, outcome is still dismal. In particular, therapeutic stratification remains suboptimal, which is largely attributed to the clinical and molecular heterogeneity of AML.

**Aims:** To better characterize and study this heterogeneity, we developed an in vivo model of AML using patient derived xenografts (PDX).

**Methods:** Primary AML were isolated from the bone marrow and peripheral blood of AML patients following informed consent and under approval from the UK National Research Ethics Service (LRCEref07/H0310/146). Differential expression analysis of RNA sequencing data (DESeq2) was used to construct a consensus model of BM-MSC AML-MIA blasts. From this model, we identified 924 down-regulated in AML derived BM-MSC and 201 up-regulated. The aim of the present study is to determine the role of hypoxia in regulating MIF signalling in AML.

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of B7 molecules in MOLM-13 cells. Our results will contribute to the understanding of MOLM-13 cell-mediated T-cell activation. Crosslinked 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% pAkt and pErk 7.9 and 7.6, n=3) compared to crosslinked CD3/CD28 antibodies (mean% pAkt and pErk 34.6 and 34.6, n=3). However, the combination of AMG 330 and CD28 increased the amount of phosphorylated proteins (mean% pAkt and pErk 11.6 and 11.1), but not to the level achieved by CD3/CD28 stimulation. In the absence of target cells, no Akt phosphorylation was observed upon incubation with AMG 330, suggesting a highly target cell dependent T-cell activation (mean% pAkt with vs without target cells: 0.8 vs 7.9).

Summary/Conclusions: Our data support the hypothesis that costimulation influences the susceptibility of target cells to lysis by T-cell recruiting antibody constructs. Currently, we are validating our results in a larger cohort using T-cells from pediatric patients with AML, where often limited research material is available. Methods: For three pediatric AML patients with mutations in the genes NPM1 and/or FLT3, we performed single cell sorting for CD34+ CD38- AML blasts by FACS and subsequently whole genome amplification (WGA) using the REPLiSING single cell DNA isolation kit (Qiagen). The amplification of single cell DNA and analysis of the corresponding bulk bone marrow was analysed by exome sequencing (WES). Bulk DNA was additionally evaluated by an amplicon-based sequencing approach targeting 54 genes (TruSight Myeloid Panel, Illumina) associated with myeloid malignancies.

Results: The analysis revealed that the median read coverage obtained in the WES of the five DNAs amplified from the single CD34+ CD38- cells and in the corresponding bulk DNAs from the bone marrow of all three patients was comparable for three out of the five single cells. For three amplified single cell genomes, between 92 and 98% of all reads could be mapped to the human genome, however the median coverage for the two failed single cells was 0. For validation of the WGA quality from single LSC DNA, data from 50 out of 54 genes analyzed by both sequencing approaches, WES and TSM Panel, were available for all three patients. The majority of variants detected in the WES bulk data could consistently be found at a comparable variant frequency in the TSM panel data. The variant frequencies in the single cell data from WES were more variable and more variants could not be detected in the TSM panel data derived from bulk DNA. We were able to detect n=79 (66%) out of n=121 somatic variants (SNVs, InDelS) present in the patients' AML blasts with all three sequencing approaches. WES readily identified n=103 (85.1%) and n=93 (78.9%) of all 121 variants in the bulk and single cell DNA, respectively. Only n=4 (3.3%) variants were not detected by WES at all. We were able to retrace the NPM1 and FLT3 mutations for each of the three patients in the targeted sequencing approach. However the NPM1 mutations and one FLT3 ITD could not reliably be called in the WES approach due to insufficient coverage.

Summary/Conclusions: In summary, WES of amplified single cell DNA is an excellent discovery tool also in pediatric AML for detecting unique changes in potential LSCs that should be validated by targeted sequencing approach with sufficient read counts for finding of rare events.
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 leukemia (AML) despite a promising response to induction chemotherapy. Emerging evidence suggest the roles of autophagy, a self-eating process contributing to chemoresistance of leukemias. We previously demonstrated that miR-10a, highly expressed in a subgroup of AML harbouring NPM1 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 leukemia.

Methods: Apoptosis and proliferation in miR-10a inhibited and overexpressed leukemia 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 mono-dansyl-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 AP2-gamma (TFAP2C). The inhibition of either miR-10a itself or CDKN1A by siRNA treatment inhibited apoptosis induced by serum starvation, treatment with autophagy inducer, mg132 or p35 stabiliser, Nutlin3a.

Summary/Conclusions: The data suggests miR-10a as an important regulator of autophagy and apoptosis in AML. Future studies need to be performed to examine the molecular mechanism 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.

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 HIF and 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 anthracyclines. 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 multivariable regression and Cox Hazard Ratio (HR) model were performed. Correlation between variables was assessed with Fisher’s exact test.

Results: Autozygous mutations (gene group 2; 12 of 14) had a KRAS gene (4 out of 24) that correctly identified a mutation in normal peripheral blood and bone marrow samples. Somatic mutations were found in 107 cases with the TET2 and GATA2 mutations on outcome.

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Background: The NGS-based MSI screen showed that 1,303 samples did not have MSI. The 64% of the remaining 200 samples (64%) had 2-4 mutations and the 2 most highly mutated samples had only 9 mutations each. Since any putative MSI+ sample would harbor tens if not hundreds of mutations, these data support the absence of MSI in all samples. Finally, because it has been proposed that t-AML might be more prone to MSI than de novo AML, we performed PCR-based MSI detection on an additional 23 t-AML cases, and found all were MS stable.

Summary/Conclusions: The absence of even a single MSI case within this large cohort provides strong evidence that MSI is non-existent in AML.

E884 SY-1425, A POTENT AND SELECTIVE RARA AGONIST, REPROGRAMS AML CELLS FOR DIFFERENTIATION ALONG DISTINCT LINEAGES, UNCOVERING PD MARKERS FOR CLINICAL STUDIES

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Background: SY-1425 (famotibartone) is a potent and selective agonist of the retinoic acid receptor alpha (RARA) transactivation factor (TF), currently in a bioinformatics-driven Ph2 clinical trial in AML and MDS patients (NCT02807558). A subset of AML and MDS has been found to have RARα pathway activation characterized by a large enhancer at the RARA locus (RARA-high) and/or upregulation of IRF8, a TF associated with RARα signaling, forming the basis of the RARA-high sensitive tumor identification.

Aims: We sought to understand how SY-1425 agonism of RARα 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 RARα, and ATAC-seq with or without SY-1425 treatment.

Cell surface marker changes were assessed by flow cytometry.

Results: A subgroup of the patient samples was defined by an SE driving RARA which co-occurred with SEs driving FOS and JUNB, or IRF8, FOS and JUNB form the AP-1 heterodimeric TF known to promote an immature cell state and the interferon regulatory factor 8 (IRF8) pathway has been implicated in AML pathogenesis. Previously reported crossstalk between INF and retinoic acid signaling was supported by the strong induction of interferon gene sets by SY-1425 in IRF8-high AML models. We found that each AML cell line had distinct compositions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocyctic, macrophage, dendritic, and granulocytic cell types. While APL has been directed 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 consisting of RARA-high AML models with observed epigenomic alterations including CD11b, CD11c, CD66b, 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 RARα were associated with greater responsiveness to SY-1425 as were enhancers bound by other TFs involved in myeloid differentiation. The accessibility of RAR elements and IRF motifs were increased in IRF8-high AML models. We found that each AML cell line had distinct compositions of lineage factors consistent with cancer initiation from different stages of myeloid development. SY-1425 induced maturation features associated with monocyctic, macrophage, dendritic, and granulocytic cell types. While AML has been directed 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 consisting of RARA-high AML models with observed epigenomic alterations including CD11b, CD11c, CD66b, and CD38 upregulation.
Aims: To identify key downstream mediators of SYK signaling in AML responsible for differentiation block, proliferation and leukemic stem cell (LSC) maintenance.

Methods: AML cell lines (KG1, MOLM14) or bone marrow primary AML blasts, were incubated 24h with R406 (1μM, 4 μM) or vehicle. Activity of SYK, ERK, STAT5 was assessed by western blot and/or intracellular phospho-flow. Proliferation was determined by the MTS assay in the presence of R406. Flow cytometry determined cell surface expression of CD25, cell number, and apoptosis. Activity toward LSC was assessed using leukemia cell line with stem cell properties (TEK), ROS level, mitochondrial mass. DNA damage and telomere length were assessed by DCF, Mitotracker Green FM staining. Expression of MYC, TFAM, NFR1, TF2, EF-Tu were assessed by western blot and/or q-PCR.

Results: To identify downstream mediators of SYK in AML, we tested the activity of key signal molecules in KG1 and MOLM14. AML cells exhibited basal R406 reduced pERK and pSYK, whereas R406 reduced MYC, transcription factors associated with LSC properties and pSTAT5, with reduced proliferation, clonogenic potential, induced differentiation and apoptosis. R406 reduced nuclear and cytosolic expression of MYC and proliferating cell nuclear antigen (PCNA). Inhibition of pERK induced dose-dependent apoptosis in LSC-enriched TEX cells. Since high pSYK is associated with upregulation of MYC and increased expression of MYC and its targets that drive mitochondrial biogenesis is a characteristic feature of LSC, we hypothesized that R406 depletes LSC by reducing mitochondrial biogenesis/oxidative phosphorylation (OXPHOS). In TEK and primary CD34+ AML blasts, R406 reduced expression of MYC, transcription factors associated with mitochondrial biogenesis (NRF1, TFAM, EF-Tu, NRF2), and lowered cellular and mitochondrial mass.

Summary/Conclusions: Taken together, we found that SYK inhibition obviates differentiation arrest imposed by ERK activity, and reduces clonogenic potential via decreased STAT5 activity. Moreover, we show that pSYK is required to sustain MYC expression and mitochondrial biogenesis/OXPHOS, a key feature of LSC in AML.

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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 and their clinical outcomes, has not been thoroughly assessed.

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 multi-amplicon targeted panel sequencing on bone marrow samples of 91 patients diagnosed with APL (PETHEMA LPA99/2000). Libraries were prepared by the TruSight Myeloid Sequencing Panel (Illumina), and 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

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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.Ser65Arg & p.Gln61Arg). Therefore, we could identify a small subgroup of patients at a very high risk of relapse (RFS at 5 years, 25% vs 100%, p<0.001).

**Aims:**
In this study, our comprehensive analyses of PDCD1 (PD-1), CD274 (PD-L1), TP53 and miR-34a expression in AML patients shed new light on the complex regulation of PD-1/PD-L1 axis during development of this disease.

**Methods:** We performed analysis of TP53, CD274 and miR-34a expression in 197 AML patients available from The Cancer Genome Atlas (TCGA) database. Moreover, we assessed mRNA expression of PDCD1 in independent cohort of 54 AML, 62 MDS and 8 s-AML patients samples using qRT-PCR method. For miRNA analysis, CD33+ cells from 29 AML patients were isolated and miR-34a expression was analysed. We also characterized several SNP for PDCD1 that demonstrate relevant associations with a higher risk of developing autoimmune diseases: PD-1.1, PD-1.3, PD-1.6, PD-1.7, PD-1.9 (rs2227981), PD-1.6 (rs10204525), PD-1.7 (rs41386349), PD-1.9 (rs2227982) in 54 AML, 62 MDS and 100 HVs samples.

**Results:** We observed significant different expressions of PDCD1 expression in groups of 54 AML, 62 MDS, 8 s-AML patients compared to HVs. TCGA data analysis showed that CD274 expression was elevated in groups with TP53 mutations compared to unmutated TP53 group (p<0.001). We also found negative correlation of TP53 and miR-34a expression with CD274 expression (p=0.02 and p=0.005, respectively). The expression of miR-34a tended to be elevated in group with high expression of TP53 compared to group with low TP53 expression (p=0.17). We have not found any differences in CD274 expression between groups with or without following mutations: IDH1, TET2, RUNX1, NRAS, CEBPA, PTPN11, KRAS, FLT3, DNTM3, NPM1 and IDH2. Patients with more than 4 recurrent mutations were characterized with higher expression of CD274 compared to group of patients with 0-3 recurrence mutations. We found that patients with >14 of all mutations had elevated expression of CD274 compared to group 0-13 mutations (p=0.06). We observed significant differences in PDCD1 expression level regarding to PD-1.1.5 polymorphism. Moreover, analysis of a PD-1.1.3 polymorphism in HVs and MDS groups revealed that genotype GG was associated with nearly fivefold lower risk of disease (OR=4.93, p=0.009). We observed significant differences in OS in AML patients in case of presence of certain genotypes of PD-1.1.6. Genotype AA was significant associated with higher risk of shorter OS compared to the rest of the genotypes (58 vs 333 days, HR=35; p=0.001).

**Summary/Conclusions:** In summary, the present study shows that the status of NRAS and FLT3 genes could be used as genetic markers for prognosis in APL, especially in the intermediate and low-risk groups, allowing a more accurate patient risk classification. Our data suggests the need to search for new mutations required for progression in APL, in order to benefit from a change in post-remission therapy.

**E888**
**ANALYSIS OF THE PD-1/PD-L1 AXIS POINTS TO ASSOCIATION OF UNFAVORABLE RECURRENT MUTATIONS WITH PD-L1 EXPRESSION IN AML.**
K. Giannopoulos1,2,*, M. Zajac1, J. Zaleska1, A. Dolnik3, A. Siwiec4, O. Jankowska-UNFAVORABLE RECURRENT MUTATIONS WITH PD-L1 EXPRESSION IN change in post-remission therapy.

**Background:** Programmed death ligand-1 (PD-L1) is regulated through miR-34a molecules in AML patients. Moreover, Cortez et al. for the first time identified novel, complete mechanism of PD-L1 regulation by p53 via miR-34a in non-small cell lung cancer (NSCLC).

**Aims:** In this study, our comprehensive analyses of PDCD1 (PD-1), CD274 (PD-L1), TP53 and miR-34a expression in AML patients shed new light on the complex regulation of PD-1/PD-L1 axis during development of this disease.

**Methods:** We performed analysis of TP53, CD274 and miR-34a expression in 197 AML patients available from The Cancer Genome Atlas (TCGA) database. Moreover, we assessed mRNA expression of PDCD1 in independent cohort of 54 AML, 62 MDS and 8 s-AML patients samples using qRT-PCR method. For miRNA analysis, CD33+ cells from 29 AML patients were isolated and miR-34a expression was analysed. We also characterized several SNP for PDCD1 that demonstrate relevant associations with a higher risk of developing autoimmune diseases: PD-1.1, PD-1.3, PD-1.6, PD-1.7, PD-1.9 (rs2227981), PD-1.6 (rs10204525), PD-1.7 (rs41386349), PD-1.9 (rs2227982) in 54 AML, 62 MDS and 100 HVs samples.

**Results:** We observed significant different expressions of PDCD1 expression in groups of 54 AML, 62 MDS, 8 s-AML patients compared to HVs. TCGA data analysis showed that CD274 expression was elevated in groups with TP53 mutations compared to unmutated TP53 group (p<0.001). We also found negative correlation of TP53 and miR-34a expression with CD274 expression (p=0.02 and p=0.005, respectively). The expression of miR-34a tended to be elevated in group with high expression of TP53 compared to group with low TP53 expression (p=0.17). We have not found any differences in CD274 expression between groups with or without following mutations: IDH1, TET2, RUNX1, NRAS, CEBPA, PTPN11, KRAS, FLT3, DNTM3, NPM1 and IDH2. Patients with more than 4 recurrent mutations were characterized with higher expression of CD274 compared to group of patients with 0-3 recurrence mutations. We found that patients with >14 of all mutations had elevated expression of CD274 compared to group 0-13 mutations (p=0.06). We observed significant differences in PDCD1 expression level regarding to PD-1.1.5 polymorphism. Moreover, analysis of a PD-1.1.3 polymorphism in HVs and MDS groups revealed that genotype GG was associated with nearly fivefold lower risk of disease (OR=4.93, p=0.009). We observed significant differences in OS in AML patients in case of presence of certain genotypes of PD-1.1.6. Genotype AA was significant associated with higher risk of shorter OS compared to the rest of the genotypes (58 vs 333 days, HR=35; p=0.001).

**Summary/Conclusions:** In summary, the present study shows that the status of NRAS and FLT3 genes could be used as genetic markers for prognosis in APL, especially in the intermediate and low-risk groups, allowing a more accurate patient risk classification. Our data suggests the need to search for new mutations required for progression in APL, in order to benefit from a change in post-remission therapy.
specifically evaluated the impact of in vitro exposure of a barcoded AML cell line (HEL) to chemotherapy regimen (doxorubicin (DOX) and/or cytarabine (CYT)) and/or the hypomethylating agent decitabine (DCT) by comparing the barcode composition of the tumor population recurring after each therapy, versus non-treated (NT) controls. By comparing the barcode architectures between parallel replicate cultures for each therapy, we could further delineate whether AML relapse was driven by predetermination of unique barcodes found in multiple replicates or stochastically selected (if mainly diverse barcodes in each replicate) cells in response to each treatment regimen.

Results: Only treatment regimens containing DOX caused marked decreases in HEL cell numbers and barcode architectures diverging strongly from the non-treated controls. Replicate AML cultures regressing 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 a minor impact (<4-fold) on cell number (i.e. yielded similar single-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 regiments. Importantly, this cell population changing by the reversion of resistance to chemotherapy re-treatment, which the DOX-containing treatment regiments 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/dis-ease 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 kinship-dependent aspects that go beyond the genetic level. Critically, these studies directly provide the rationale for combining standard chemotherapy with administration of hypomethylating drugs to target AML. The mechanism is prevention of the development of chemotherapy resistance (mediated by selective 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 sur-vivable/ 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) could improve prognostication of a possible relapse during the remission. Traditional methods for measuring minimal residual disease (MRD) in AML, such as real-time PCR and multiparametric flow cytomtery (MFC) are associated with high technical complexity, low accuracy 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 techniques for measuring MRD levels are insufficient.  

Aims: To detect minimal residual disease in AML follow-up sample using high-throughput sequencing as a standard and accurate technique.  

Methods: We studied 54 gDNA bone marrow follow-up samples (27 after induction, 10 after first consolidation, 17 after second consolidation) from 30 AML patients treated according to PEThema AML clinical protocols and with DNA samples at diagnosis. All patients had achieve CR at the moment of MRD assessment. We developed a custom-targeted sequencing panel of 32 genes (ion Torrent Proton System-Thermo Fisher) for mutation (SNV and/or InDels) detection at diagnosis sample. From the 32 genes, we use specific primers to amplify the specific region of the four most frequent alterations at diagnosis (Samples at follow-up: FLT3n-ITD n=2, NPM1n=14, 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 sensitivity 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 10-5-technical sensitivity) by ROC curve with a sensitivity of 0.5 for DFS and 0.571 for OS, and a specificity of 0.92 for DFS and 0.897 for OS; thereby this result above this level was considered as MRD positive. DFS (Disease Free Survival) and OS (Overall Survival) rates in this group were 29.9% and 24.1%, respectively; positive MRD sample was independent marker associated with shorter DFS (p=0.002, HR=0.33, 95% CI:1.60-33.51) and OS (p=0.002, HR= 8.33, 95% CI:1.87-37.15) (see figure 1). These results support the usefulness of MRD evaluation in patients with AML by NGS in the context of molecular biology studies.

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/dis-ease 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 kinship-dependent aspects that go beyond the genetic level. Critically, these studies directly provide the rationale for combining standard chemotherapy with administration of hypomethylating drugs to target AML. The mechanism is prevention of the development of chemotherapy resistance (mediated by selective 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 sur-vivable/ repeatedly clinically manageable episodes of a type of chronic leukemic disease.

E890  
THE ROLE OF MYELOID-DERIVED SUPPRESSOR CELLS-LIKE BLASTS WHICH SUPPRESS T CELL PROLIFERATION IN LEUKEMIC CELL GROWTH  
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Background: Myeloid-derived suppressor cells have an ability to suppress T-cell function and have been known to facilitate tumor growth. We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.  

Aims: We elucidated the immune suppressive function of leukemic blasts which resembled MDSC phenotype and their role in the growth of leukemic cells.  

Methods: CD11b+CD33+HLA-DR-blast (MDSC like blast) were isolated using flow-cytometry from bone marrow mononuclear cells of primary acute myeloid leukemia (AML) patient samples. CD14, CD15, Arg1 and iNOS expression were checked by flow-cytometry to identify the phenotype of MDSC like blast. To evaluate the ability of MDSC like blasts to suppress T cell proliferation, CD8+ T cells from healthy donor and MDSC like blasts were co-cultured with a ratio of 1:1 without phytohemagglutinin A 10ug/ml. T-cell proliferation was measured by carboxyfluorescent diacetate succinimidyl ester dilution assay after 3 days of culture. Then, various leukemic cell lines were co-cultured with jurkat T cells and/or MDSC like blasts at a leukemic cell line:Jurkat cell T: MDSC like blast ratio of 4:4:1. The effect of Jurkat T cells and MDSC like blasts on the proliferation of leukemic cells was assessed by the CCK-8 assay after 1 and 3 days of culture.  

Results: MDSCs like blast can be divided into two subtypes, monocyteic subgroup expressing CD14 and granulocytic subgroup expressing CD15, and CD14 expression was more frequent than CD15. Myeloid-derived suppressor cells showed higher expression of Arg1 (77.1% vs 38.5%, P<0.001) and iNOS (33.0% vs 1.1%, P=0.0001) compared to non-MDSC-like blasts. CD8+ T cell proliferation induced by PHA was significantly suppressed when co-cultured with MDSC-like blasts compared to without them. Among the various leukemic cell lines, the proliferation of NB4 cells were significantly suppressed when co-cultured with jurkat T cells on day 3 (NB4 23.49±6.26% of control, NB4+jurkat 12.62±3.92%, P<0.01). The decreased proliferation of NB4 cells was partially recovered after 3 days of co-culture with MDSC-like blasts (NB4+jurkat 12.62±3.92%, NB4+jurkat+MDSC like blast 18.71±6.19, P=0.022).

Figure 1.  
Summary/Conclusions: High-throughput NGS is a technique with the capacity to measure, identify and classified MRD levels. In fact, NGS MRD evaluation has a better DFS and OS prediction than other traditional methods. Implementation of NGS technique on MRD detection could help to anticipate to disease progression.  

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Summary/Conclusions: CD11b+CD33+HLA-DR: MDSC-like blasts subpopulation which expressed the INOS and Arg1 existed in AML, and showed ability to suppress the T cell proliferation. MDSC-like blasts partially restored the suppressed leukemic cell growth of NB4 cells by jurkat cells. MDSC-like blasts might play a certain role in immune-escape mechanism of AML.

E893

GENERATION OF NEW CELLULAR MODELS FOR THE STUDY OF PEDIATRIC NON DOWN SYNDROME ACUTE MEGAKARYOBLASTIC LEUKAEMIA 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 megakaryblast progenitors 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 put these two mutation types to the test.

Results: We developed AMP-based Archer®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. MBG adapters ligated to RNA and DNA fragments prior to amplification enable relative gene expression and CNA analysis.

Results: We show instances of gene fusion detection from open-ended amplification of AML samples, including AML-ETO, RUNX1-RUNX1T1 and TEL-AML1. We found unclassifiable leukemia. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected 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 CEPBPα. Finally, MBG used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B-Cell Lymphoma subtypes in a small cohort of samples.

Conclusions: Our results 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.

E895

ASXL1 MUTATIONS IN AML ARE ASSOCIATED WITH SPECIFIC CLINICAL AND CYTOGENETIC CHARACTERISTICS

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Background: Mutations of ASXL1 are considered early founder events in AML development. They are included in the definition of the "chromatin-splice-some" genomic class of AML and among the high risk genetic prognosticators in the 2017 ELN recommendations.

Aims: We aimed to study the frequency of ASXL1 mutations in a cohort of newly diagnosed AML patients and to look for correlations with conventional cytogenetic findings and baseline characteristics.

Methods: Three hundred and sixty AML patients diagnosed between 2005 and 2014 were studied. Conventional cytogenetic analysis was performed on unstimulated bone marrow cells cultured for 24 and 48 hours. Molecular analysis of ASXL1 exon 12 mutations was performed by PCR and subsequent direct sequencing in diagnostic bone marrow or peripheral blood samples.

Results: Median age of the whole cohort was 63 years (11-95) and 56% of patients were male. Eighty two patients (22.8%) had secondary AML (sec-AML) with prior diseases being MDS (63), CMML (4), PV/ET (9), MF (2) and CML (4). Karyotypic analysis was successful in 352 (97.7%) AML samples of which 252 (71.6%) exhibited clonal karyotypic abnormalities. ASXL1 mutations were significantly more frequently detected in 52 patients (14.4%). The most common mutation was c.1394dupG in 44/52 (84.6%). ASXL1 mutated patients were significantly older with median age 72 vs 61.5 years in the unmutated (p=0.0001). Three of 61 patients (4.9%) aged ≤40, 10/97 aged 41-60 (10.3%) and 39/198 aged >60 (19.7%) were mutat-

E894

CHARACTERIZATION OF HEMATOLOGIC MALIGNANCIES WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING

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Background: Hematologic malignancies can be driven by a diversity of mutation types, including single nucleotide variants, copy number variants, gene fusions, insertions and deletions and changes in gene expression profiles. However, comprehensive detection of these mutation types from a single clinical sample is challenging, as specific assays are required to detect each mutation type. Next-generation sequencing (NGS) enables comprehensive detection of all mutation types from whole genomes and transcriptomes. However, low detection sensitivity, high input requirements and high costs render these approaches impractical for routine detection of mutations from clinical sample types. Anchored Multiplex PCR (AMP™) is a target enrichment strategy for NGS that uses molecular barcoded (MBC) adapters and unidirectional gene-specific primers (GSPs) for amplification.

Aims: Our goal was to develop AMP-based NGS assays to simultaneously detect multiple mutation types from DNA and RNA, as well as relative gene expression levels and copy number alterations (CNA) in hematologic malignancies. In particular, we sought to develop methods to detect novel gene fusions, internal tandem duplications (ITDs) and mutations in CEPBPα.

Methods: We developed AMP-based Archer®VariantPlex™ and FusionPlex® assays to enable NGS-based detection of mutations from DNA and RNA, respectively. Open-ended amplification permits identification of novel gene fusions with FusionPlex and complex mutation types such as ITDs with VariantPlex assays. MBC adapters ligated to RNA and DNA fragments prior to amplification enable relative gene expression and CNA analysis.

Results: We show instances of gene fusion detection from open-ended amplification of AML samples, including AML-ETO, RUNX1-RUNX1T1 and TEL-AML1. We found unclassifiable leukemia. Furthermore, unidirectional GSPs provided bidirectional coverage of a BCR-ABL1 fusion, which was detected with reads originating from ABL1 as well as BCR. Using our optimized bioinformatics algorithm and the VariantPlex assay, we accurately and reliably detected 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 CEPBPα. Finally, MBC used in AMP enabled NGS-based expression profiling for identification of Diffuse Large B-Cell Lymphoma subtypes in a small cohort of samples.

Conclusions: Our results 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.

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mutations. Moreover, ASXL1 mutations were detected in 3 of 12 patients with aberrations (25%), 2/9 (22%) with trisomy 15, 2/11 (18%) with t(5;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 as del(7q) or -5/del5q predicted for lower frequency (OR 0.32, 95% CI 0.13-0.75).

Summary/Conclusions: Our results support the association of ASXL1 mutations in AML with advancing age and sec-AML. Association with trisomy 8 did not retain significance in multivariate analysis. Chromosome 11 aberrations emerged as the independent predictor. Despite the strong link with secondary AML (majority of cases post MDS), our data show inverse relationship with -7/del(7q) or -5/del5q. In addition, ASXL1 mutations were not positively associated with MDS-related cytogenetic abnormalities, complex or monosomal karyotypes.

E896
Abstract withdrawn.

E897
A COMPREHENSIVE DNA TEST FOR THE DETECTION OF TRANSLOCATIONS IN ACUTE LEUKEMIA
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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(15;11), t(11;19)(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 marrow of 36 patients suspected karyotyping, FISH and RT-PCR were taken for routine diagnostic diagnosis (karyotyping, FISH and or RT-PCR) and TLA. Sample analysis was performed randomized and blinded. TLA outcome was then compared with results from routine genetic tests, can establish whether the TLA panel is applicable as a routine procedure.

E898
ALTERATIONS IN NECROPTOSIS PATHWAY AFFECT PROGNOSIS OF PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background: Necroptosis is a type of necrotic cell death involving several genes transcription and activation of molecular mechanisms as death receptors, interferon, toll-like receptors, intracellular RNA and DNA sensors. The process is leading by the family of receptor-interacting protein kinase (RIPK3, RIPK2, RIP1K) 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 18 patients presented a loss of RIPK1 or MLKL (nobody presented losses in RIPK3/RIPK2) and 13/18 patients were older than 65 years old. The Overall Survival (OS) of patients with alterations in these genes is significantly lower than control group, with a median OS of 3 month respectively (p<0.001). With Fisher Exact Test we further demonstrate that copy number loss of RIPK1 or MLKL are associate to loss of TP53 or FANCA genes, complex karyotype and advanced age. COX-Hazard Ratio model with RIPK1 or MLKL loss, BRCA1 loss, TP53 mutation, FANCA loss, secondary disease and diagnosis karyotype considered as categorical variable show that necroptosis deficiency (HR 1.98, CI 95% 1.04-3.78) TP53 mutation, and secondary AML are independent negative prognostic factors in an optimal model.

Summary/Conclusions: Our study shows that losses in necroptosis pathways are an uncommon alteration in AML prevalent in old population. Moreover, we hypothesize that the loss of genes involved in necroptosis could be a real mechanism of tumor immune-escape and could be a rational to select patients that high probability to be resistant at chemotherapy promoting ICD mechanism.

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E899
NGS ANALYSIS AND IMPACT OF VARIANT ALLELIC FREQUENCY AT RELAPSE AND REFRACTORY STATUS IN AML PATIENTS
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Background: A high number of patients with acute myeloid leukemia (AML) present resistance at treatment, which is associated with clonal persistence or evolution. The generation of high-depth sequencing data allowed to quantify variant allelic frequencies (VAF) and permitting estimation of the size of tumor clonal populations in each AML sample, and to perform an estimation of clonal evolution at relapse or refractory case according at diagnostic.

Aims: To evaluate the predictive impact of the fluctuation Variant Allelic Frequency in resistance to treatment cases in AML.

Results: FLT3, JAK2, KIT, LNK or/and MPL) play a critical role in resistance status, as RUNX1 correlates with primary refractory samples, p=0.015; mutations in JAK2, KMT2A or SF3A1 shows significant ΔVAF decrease in primary tumor-refractory (n=8) and primaty tumor-relapsed (n=17) samples pairs from 25 AML patients treated according PETHEMAL 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: Mutations in signalling pathway (EPOR, FLT3, JAK2, KIT, LNK on/and MPL) and GTPases pathway (KRAS, NRAS, HRAS) present significant ΔVAFs increases in relapse samples, p<0.05 and p=0.039 respectively. See figure 1. Furthermore, mutations in IDH2, JAK2 or KRAS show ΔVAF trend increases. Also, mutations in signalling pathway shows significant ΔVAF decrease in primary refractory samples, p=0.015; mutations in JAK2, KMT2A or SF3A1 shows ΔVAF trend decreases. Regarding to mutational profile we found significant ΔVAF decrease in primary tumor-refractory samples. No correlation was found between VAF and ΔVAF trend decreases. No correlation was found between VAF and% blasts, nor did VAF fluctuation with blasts fluctuation.

Summary/Conclusions: These results show VAF increases of specific mutations as KIT correlates with relapse status. However, in this cohort new specific mutations were not found neither VAF increases, even the opposite being as VAF decreases of specific mutations as RUNX1 correlates with primary refractoriness status. Furthermore, the variable frequency signalling pathway (EPOR, FLT3, JAK2, KIT, LNK on/and MPL) play a critical role in resistance status, increasing variant allelic frequencies of mutations in relapse and decreasing in refractoriness.

This work was supported by the grant: PI13/02387.

E900
PRECLINICAL EVIDENCE THAT TRAMETINIB ENHANCES THE RESPONSE TO TYROSINE KINASE INHIBITORS IN ACUTE MYELOID LEUKEMIA
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Background: Acute Myeloid Leukemia (AML) is the most common type of acute leukemia in adults and the second in children in whom overall survival is less than 35% and 60% respectively. Activating mutations of FLT3 are now recognized as the most common molecular abnormality in this disease, and the poor prognosis of patients harboring these mutations renders FLT3 an obvious therapeutic target. Although different tyrosine kinase inhibitors (TKI) have been used for this purpose, their ability to extend progression-free and overall survival is limited by drug resistance. This strategy could be improved by rationally combining TKIs with other agents. In this work, we have explored bone marrow samples from a FLT3-AML patient before and after TKI treatment by phosphoproteomics and observed enhanced activity of Ras-Raf-MEK-ERK1/2 pathway as a possible mechanism for TKI resistance.

Aims: To validate the role of ERK1/2 during TKI treatment in vitro and ex vivo and to search suitable combinations that allow overcome/avoid resistances in preclinical models of the disease.

Methods: Resistance mechanisms were studied in vitro in MOLM13 (FLT3WT/ITD) after generating resistance by two different methods: by subculturing with increasing doses of sorafenib or by treating them with high doses of sorafenib, and recollecting alive proliferative (resistant) cells after CFDA and Annexin labeling. Phosphoproteomic analyses were carried out by LC-MSMS after IMAC enrichment or by western blot techniques. Drug sensitivity assays with trametinib (MEK inhibitor) and three TKIs (sorafenib, pazopanib, midostaurin) were read after 48 hours or 72 hours of treatment in vitro or ex vivo respectively. The efficacy of the combinational treatments was characterized by cell viability assay using WST8, and analyzed with Graphpad Prism software. Synergy effects were measured with CalcuSoft software.

Results: As it is presented in figure 1, ERK1/2 pathway was more activated after TKI treatment in the FLT3-AML patient during sorafenib-resistance development. The same fact was confirmed in MOLM13 sorafenib-resistant culture and in living proliferative cells recollected after sorafenib treatment. Different doses of trametinib, sorafenib, pazopanib and midostaurin in monotherapy were tested in MOLM13 cell line determining their IC50 values. Synergy effects of combining trametinib with the three TKIs were analyzed with CalcuSoft software and normalized isobolograms were represented. All combinations showed CI values less than 0.5 (CI<0.5). The inhibition levels of four pathways (PI3K, STAT5, ERK1/2 and MAPK14) implicated in TKIs resistance were studied by western blot and the combination of midostaurin plus trametinib was the only one that inhibited all of them. Trametinib efficacy in the MOLM13 sorafenib-resistant culture was evaluated and confirmed that remained effective. Trametinib plus midostaurin combination was tested in OCI AML-3 cell line (FLT3WT/ITD) showing high efficacy and strong synergy (CI<0.5) too. Finally, we have assayed these drugs ex vivo in three AML samples showing the same effectiveness as in vitro, with IC50 values ranging from 0.2µM to 0.9 µM for midostaurin and 3 nM to 29 nM for trametinib and CI values less than 0.5.

Summary/Conclusions: In conclusion, we provide preclinical evidence that combining a TKI, especially midostaurin, with a MEKI, such as trametinib, is a rational and efficacious treatment regimen for AML. As trametinib has previously shown good results when combined with pazopanib in clinical trials for other kinds of tumors, we expect similar results in AML.
IDENTIFICATION OF NOVEL THERAPEUTIC DRUGS IN DISTINCT PEDIATRIC AML SUBTYPES BY TARGETING EPIGENETIC REGULATORS

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Background: Treatment protocols for pediatric acute myeloid leukemia (AML) are chemotherapy-based, including high-dose cytarabine. While >90% of patients reach clinical remission, there is still a high relapse rate of ~30%, with overall survival rates of 60-70%. Therefore, better risk-classification at diagnosis and alternative treatment strategies are warranted. There is increasing evidence that epigenetic deregulation is involved in the initiation and progression of cancers, including AML. Epigenetic processes are required for hematopoiesis and epigenetic regulators are frequently translocated (MLL) or mutated (EZH2) in AML. Following this, deregulated epigenetic pathways could be used for targeted therapy and provide an alternative approach to improve pediatric AML therapy.

Aims: To identify new therapeutic drugs in pediatric AML by using an 80-compound screen containing inhibitors of epigenetic regulators, including histone writers (which deposit post-translational modifications (PTMs) on histones), readers (binding of PTMs) and erasers (removal of PTMs).

Methods: Cell lines used in this study are THP-1 (t(9;11)), Kasumi-1 (t(8;21)) and CMK (Down’s syndrome with GATA1 mutation), reflecting distinct pediatric AML entities and a differential response to treatment with cytarabine. Cells were treated for 72hrs followed by analysis of cell viability and apoptosis based on Hoechst, Draq7 and Calcein Green staining. The effect of three candidate compounds were further investigated in triplicates at different concentrations for their effect on cell viability (Annexin V/FITC staining), cell cycle, morphology, and apoptosis. AML-blast cells, normal myeloid precursor cells and cord blood, and pediatric AML patient cells representing distinct AML subtypes.

Results: From the 80 epigenetic compounds tested in THP-1, Kasumi-1 and CMK cells, we observed significant effects following treatment with the HDAC 4/5 inhibitor LMK235, the pan-HDAC inhibitor NSC3852, and the pan-bromodomain inhibitor Bromosporine. Dose-response curves showed differential cytotoxicity of the compounds and suggested LMK235 as most effective. Cell proliferation was inhibited by LMK235 at an IC50 of 0.1uM, 0.13uM and 0.425uM in Kasumi-1, CMK and THP-1, respectively. While inhibition by LMK235 resulted in an immediate response of apoptosis, Bromosporine-treated cells retained G1 between 72 hours, and, interestingly, treatment of LMK235-treated cells resulted in an increase of cells in S-phase and G2/M. Among the differential effects of the compounds in the cell lines, we also observed differences in sensitivity. In line with previous studies, THP-1 cells were more resistant, illustrated by a 10-fold increase in concentration required for NSC3852-induced apoptosis. Interestingly, upon treatment with Bromosporine, Kasumi-1 and CMK cells showed a similar response, while Kasumi-1 cells were significantly more sensitive to NSC3852-induced effects. These data are currently validated in pediatric AML patient cells.

Summary/Conclusions: Treatment of three distinct pediatric AML cell lines with the epigenetic compounds LMK235, NSC3852 and Bromosporine resulted in cell line-specific effects, regarding compound sensitivity, and compound specific effects, including cell cycle regulation and induction of apoptosis. Our data suggest a potential role for epigenetic compounds, with specificity for molecular subtypes, in the treatment of clinically and biologically distinct pediatric AML subtypes.

Combination of Interferon-alpha and Valproic Acid in Acute Myeloid Leukemia Cells In Vitro and In Vivo

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Background: Interferon alpha (IFNα) monotherapy is effective in selected myeloid neoplasias and is proposed to act through mechanisms that may be additive to the action of valproic acid (VPA), a histone deacetylase (HDAC) class I and IIa inhibitor with effect in approximately 20% of acute myeloid leukemia (AML) patients. Both drugs are found to have direct anti-cancer effects targeting apoptosis, differentiation and proliferation, as well as indirect effects targeting the immune system.

Aims: As several IFNα formulations are commercially available, we wished to explore the differences between two such drugs, recombinant IFNα2b and human IFNα-Le, in relevance to AML treatment.

Methods: Flow cytometry and Hoechst staining was used to investigate apoptotic potential of the IFNα therapeutics, whilst phospho-flow cytometry and differences in electrophoresis in combination with mass spectrometry unraveled IFNα signaling pathways. For in vivo efficacy analyses two orthotopic rodent models were implanted with leukaemic cells, and treated with VPA, IFNα-Le or both drugs.

Results: To investigate the anti-leukemic effects of IFNα we combined the two therapeutics with VPA in vitro using the human MOLM-13 cell line (wild type for FLT3 ITD and TP53). Results showed that IFNα-Le was more efficient compared to IFNα2b in in vitro studies, whilst both drugs were less effective in combination with VPA. Investigating IFNα signaling pathways using phospho-specific flow cytometry we found IFNα2b and IFNα-Le to have an identical stimulation profile in MOLM-13 cells, except from p-STAT6 Y641 that was higher expressed by IFNα2b. The phospho-proteome was further explored using difference gel electrophoresis (DIGE) and mass spectrometric analyses to find a potential explanation to the difference in apoptosis-inducing effects between the two drugs. Here we found protein folding (LCP1, HSPA8, TCP1, CCT6A), cell stress (AKR1B1, HSP90A1) and cell death (PKM2, PARK7, HSPB1, HSPA5, ANXA5, PRDX2) to be differently regulated between IFNα2b and IFNα-Le, and also identified the presence of a clearance-dependent effect on protein expression by IFNα2b and IFNα-Le. Further, we investigated the potential synergistic anti-leukemic effects of VPA and IFNα-Le in vivo using a MOLM-13LacIimmunodeficient NOD/Scid IL2 g- orthotopic xenograft mouse model, and the
immunocompetent brown Norwegian myeloid leukemia (BNML) syngeneic rat model. VPA mono-treatment increased survival from a median of 34 days to 38 days in the MOLM-13

BNML mouse model, and from 21 days to 50 days in the BNML rat model. Additionally, the IFNo-Le (0.8x10^6 IU/kg) and VPA (400mg/kg) combination treatment indicated a tendency to increased survival in the BNML model. However, IFNo-Le monotherapy (1x10^6 IU/kg) decreased survival in the MOLM-13

model.

Figure 1.

Summary/Conclusions: IFNo 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 heterogeneous disorder defined by clonal expansion of immature myeloid cells that infiltrate bone marrow and other tissues. AML therapeutic strategies remain unchanged since 1970 and the majority of patients often eventually relapse and die due to disease progression. Tumor protein p53 transcription factor is a key regulator of several cellular pathways, such as cell cycle arrest, cell death and changes in gene expression. 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 −20°C in sterile water in a 600 μg/ml stock solution, and diluted in medium immediately before use [concentration range in use 15-60μg/ml]. Cell lines, MOLM-13 and KASUMI-1, were cultured in RPMI 1640 supplemented with 20% heat inactivated fetal bovine serum, 2mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. After 24 and 48 h of treatment MTS, Annexin-V, TUNEL, JC-1 and Active Caspase-3 assays were performed according to manufacturer’s instructions. Proteins were separated by polyacrylamide gel electrophoresis and transferred to 0.2 μm polyvinylidene fluoride membranes. Quantitative analysis was performed with Quantity One software. Statistical analysis was carried out using the paired and unpaired two-tailed Student’s t tests. p values <0.05 were considered as significant.

Results: Our data indicate that Kevetrin exposure induces cell growth arrest, a great drop of mitochondrial membrane potential and a remarkable increment of 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-L56S 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, USA) covering 54 genes with relevance in myeloid diseases. Gene variants at Variant allele frequency (VAF) of ≥10% at diagnosis and VAF of ≥1% during follow-up; both with target coverage of ≥300 reads were considered. Bone marrow (BM) or peripheral blood (PB) was obtained at presentation (BM-44; PB-1) and follow-up (BM-130). Gene variants in 95 samples from 40 MDS patients were also evaluated for progression to secondary AML. Public databases-Catalogue of Somatic Mutations In Cancer (COSMIC), dbSNP and 1000 genome (>2%) were used to classify gene variants as either Drivers (D), variants of unknown significance (VUS) and germline polymorphisms (SNP). P-value was generated with 2-tailed Fisher Exact (GraphPad Software, Inc, USA).

Results: Of 45 AML patients 19 achieved complete morphological remission (CR), 21 had a relapse and 5 had refractory disease with a median of 4 mutations/patient in each subgroup. Driver mutation was identified in 38 patients; 82% of who had persistence until clinical end-point. While 17 of 18 relapse patients retained a driver only 9 of 15 patients in remission retained it (Table 1). 8 of the 9 patients had a ‘driver with COSMIC and SNP’ (D-C/S) reference that persisted, while all ‘driver with COSMIC only’ (D-C) disappeared post-induction. This suggests that drivers with both COSMIC and SNP reference may not always contribute towards disease progression. We also found that D-C mutations persist in 85.7% of relapse patients compared to only 11% of patients in remission (P-value: 0.001). Additionally, D-C mutations were retained in all 13 relapse patients with intermediate risk cytogenetics while complete clearance was observed in all 6 patients who were in sustained remission (P-value: 0.001). Further investigation of genes with D-C/S mutation in the remission cohort (8x) revealed that 4 patients had persistent DNMT3A-2545724, 1 had DNMT3A-25457243, 2 had RUNX1-36259324/L56S and 1 had CBL-119149011. As DNMT3A mutations are considered to occur in pre-leukemic
stem cells contributing to clonal haematopoiesis (Askush et al, Nature 2014; Genovese et al, NEJM 2014); this led us to study the distribution of RUNX1 gene variants in an additional 119 AML diagnostic samples. 34 patients (21%) harboured RUNX1 mutation, of which 5 had RUNX1_L56S that were often associated with D-C mutations (4 of 5 cases). Finally, we evaluated kinetics of D-C in 40 MDS cases of which 34 had chronic MDS and 6 had secondary AML (sAML). No significant difference was observed in the number of patients with persistent D-C mutation in the 2 subgroups (chronic MDS: 16 of 19 (84.2%); sAML: 5 of 5 (100%); P-value: 1.000).

Table 1.

<table>
<thead>
<tr>
<th>AML Cohort (n=41 patients)</th>
<th>Clinical Outcome</th>
<th>Morphological remission</th>
<th>AML relapse</th>
<th>Refractory disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients in each clinical outcome category</td>
<td>19</td>
<td>21</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Mean Age (Range, in years)</td>
<td>55.8 (30-71)</td>
<td>57.3 (30-77)</td>
<td>59.8 (61-82)</td>
<td></td>
</tr>
<tr>
<td>Time to clinical end-point (Range, in months)</td>
<td>5.2 (3-15)</td>
<td>17.8 (9-66)</td>
<td>4 (3-12-22)</td>
<td></td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>Intermediate</td>
<td>12</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Poor</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Missing data</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Median no. of mutations per patient at presentation</td>
<td>15</td>
<td>18</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>No. of patients with D-C mutation that was resolved until clinical end-point</td>
<td>9 of 15 (60%)</td>
<td>14 of 17 (88%)</td>
<td>15 of 20 (75%)</td>
<td></td>
</tr>
<tr>
<td>No. of patients who related D-C mutation and acquired new D-mutations at clinical end-point</td>
<td>4 of 15 (26%)</td>
<td>14 of 17 (88%)</td>
<td>16 of 20 (80%)</td>
<td></td>
</tr>
<tr>
<td>No. of patients who lost D-C mutation and/or acquired new D-mutations at clinical end-point</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No. of patients with D-C mutation that disappeared and reappeared at clinical end-point</td>
<td>4 of 15 (26%)</td>
<td>14 of 17 (88%)</td>
<td>16 of 20 (80%)</td>
<td></td>
</tr>
<tr>
<td>No. of patients without D-C mutation at presentation</td>
<td>11 of 15 (73%)</td>
<td>12 of 17 (71%)</td>
<td>13 of 20 (65%)</td>
<td></td>
</tr>
<tr>
<td>No. of patients with intermediate risk cytogenetics and D-C mutation at diagnosis</td>
<td>6 of 15 (40%)</td>
<td>1 of 17 (6%)</td>
<td>0 of 20 (0%)</td>
<td></td>
</tr>
<tr>
<td>No. of patients with intermediate-risk cytogenetics and persistent D-C mutation</td>
<td>6 of 15 (40%)</td>
<td>1 of 17 (6%)</td>
<td>0 of 20 (0%)</td>
<td></td>
</tr>
<tr>
<td>No. of patients with persistent D-C mutation at diagnosis</td>
<td>6 of 15 (40%)</td>
<td>1 of 17 (6%)</td>
<td>0 of 20 (0%)</td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: Clearance of ‘Driver-COSMIC only’ mutations while RUNX1_L56S persists is unlikely to contribute towards disease progression in AML.

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 (allogeneic-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 allogeneic-HSCT is considered as the best therapeutic option.

Aims: The objective of this study is to determine prognostic factors and develop a prognostic score using usual mutational status, cytogenetic, histological and simple clinical variables in R/R AML patients before salvage treatments.

Methods: In this retrospective study in two hematological departments (Hospices Civils de Lyon and CHU of Toulouse), we evaluated clinical, biological, histological, cytogenetic and current mutational status of early R/R non APL AML patient between age from 18 to 70 years. Univariate and multivariate analysis were performed and we developed a prognostic score based on the independent prognostic parameters from Cox model.

Results: From January 2009 to May 2016, 58 patients presenting early relapse and primary refractory AML were analyzed. Overall Survival (OS) and Progression Free Survival (PFS) median were 9 and 2 months respectively. In univariate analysis, cytogenetic findings (unfavorable groups), unfavorable ECOG (>2), FLT3 positive status and <50% blast decrease (between induction and R/R assessment) independently predicted poor OS and were identified as significant prognostic parameters of OS (p=.037, p=.0084, p=.0452, p=.0071 respectively). In multivariate analysis, these last four criteria confirmed their worst prognostic impacts (p=.015, p=.017, p=.026, p=.015 respectively) and were used to create a five groups prognostic score. Better OS were statistically observed for patient with score 0 or 1 compared to 2, 3 or 4 (2-years OS 48% and Not Reached respectively, p=.0104) using a log-rank regression. When data were censored to allogeneic HSCT, the scoring system revealed a relevant difference with favorable OS for those with a score 0-1 compared to score 2-3-4 (2-years OS 64% and Not Reached respectively, p=.001) (Figure 3).

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 syndromes (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 [refractory/refractory (R/R) or ≤65 years until for intensive chemotherapy (IC)], or MDS failing prior therapies.

Methods: A phase I study (EUDRACT 2014-002559-24, NCT02920541) is underway to investigate S55746/BCL201 as a single agent in 5 European and Australian centers. S55746/BCL201 was initially administered in fasting conditions, once daily (21-day cycles), until disease progression, unacceptable toxicity, or investigator’s or patient’s decision. Pts giving informed consent received S55746/BCL201 at escalating dose levels according to a modified continual reassessment method for dose allocation.

Results: As of 23 February 2017, 34 pts have received S55746/BCL201 at doses ranging from 100 to 1300mg/day (median time on treatment: 43 days, range 1 to ≥374), 28 pts were R/R AML, 2 pts were elderly AML, 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 G/L (range 0-30). Among the AML cohort, European LeukemiaNet risk (Döhner 2010) was adverse in 53%, intermediate-1 in 20%, and intermediate-1 in 17%. Preliminary PK results in fasting pts showed that exposure increased linearly but with some inter-individual variability. Most common (≥20% of pts) non-hematological adverse events (AEs), all grades, included diarrhea (27%), hypokalemia (27%), nausea (21%), and vomiting (21%). The most frequent grade ≥3 AEs were hematological (anemia (35%), thrombocytopenia (32%), febrile neutropenia (21%), and neutropenia (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 8 cycles of treatment (900mg). Non-related fatal AEs were reported in 7 pts (including sepsis, hemorrhagic stroke, pneumonia, and disease progression). There were no reported episodes of clinical or laboratory TLS. No DLT was reported and MTD has not been reached. Of 26 AML pts evaluable for response (at least 1 cycle completed), one achieved a CRi (complete remission with incomplete blood count recovery lasting 10 months) and one a PR (partial remission lasting 3 months before proceeding to allogeneic stem cell transplant). In MDS, 4 out of 4 pts had stable disease (lasting 1 to ≥7 months). Bone marrow blasts decreased in 50% of all evaluable pts, with the nadir reached (87%) within the first two cycles (Figure 1).

Summary/Conclusions: Initial findings suggest that S55746/BCL201 has acceptable tolerability and clinical activity in advanced AML and MDS. Based on non-compartmental pharmacokinetic food interaction results from another study, demonstrating that S55746/BCL201 Cmax and AUC increased about 6-fold with food, dose escalation has started in patients with drug intake during a meal.

E098

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. DNM3A, IDHI, IDH2 (R140Q 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, NCI 2017).

Aims: The aim of our study was to investigate if detection of NPM1 transcripts by multiparametric flow cytometry (MFC) might represent an alternative tool to discriminate different prognosis within the NPM1 mutated AML group, in a setting where an extensive gene profiling at diagnosis or a quantitative determination of NPM1 transcripts in remission would not be available.

Methods: We analyzed a series of 69 AML patients with NPM1 mutations; all the patients were in complete remission (CR) after intensive induction cycle of EORTC-GIMEMA protocols. The frequency of NPM1 mutated cases was not different among patients below (48/142, 34%) or above (21/61, 34%) the age of 60 years, respectively. Twenty out of 65 patients (31%) carried a concomitant FLT3-ITD mutation; 51/66 (77%) NPM1 mutated cases had a normal diploid karyotype. Upon full hematological recovery after consolidation cycle, counting by MFC, ≥3.5x10^4 (0.035%) residual leukemic cells (RLCs) in the bone marrow (BM) was regarded as a condition of MRD positivity.

Results: Among NPM1 mutated patients, the rate of MRD negative CR was significantly lower (5/69, 7%) as compared to NPM1 WT ones (39/134, 29%), respectively (p<0.001). Although there was not a statistically significant difference, probably due to the low numbers, MRD negative/NPM1mut patients had a lower Cumulative Incidence of Relapse (CIR) as compared to MRD positive/NPM1mut patients (93% vs 80%). We also evaluated the impact of autologous (AuSCT) or allogeneic (ASCT) transplantation on the outcome of MRD positive/NPM1mut patients. The overall survival (OS) was significantly higher for patients submitted to ASCT (n=14) as compared to those (n=15) submitted to AuSCT (93% vs 33%, p=0.011). This was confirmed even after excluding from the analysis FLT3-ITD+ppatients. 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 NPM1mut 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.

E099

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, immune checkpoint expression of PD-1, an inhibitory receptor on the surface of T cells, can lead to dysfunction of antitumor effector cells. Recently, investigators have detected overexpression of PD-1 for patients with acute myeloid leukemia (AML) who experienced relapse following allogeneic stem cell transplantation

Figure 1.
(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<0.0001), persistence after CTx (P=0.025 and P<0.0001), and NMTR after SCT (P<0.0001 and P<0.0001). In contrast, no difference in PD-1 expression was observed between patients at time of diagnosis and patients after CTx (Figure 1). For CD4+ T cells, a significant difference was found between SCT and CTx groups, and PD-1 expression levels of groups that experienced relapse (P<0.0001) or persistence (P<0.0001) after SCT were significantly higher than those of patients in the CTx groups. PD-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=0.001 and P=0.039). Although PD-L1 and PD-L2 expression levels for patients at time of AML diagnosis did not differ from groups that experienced relapse or persistence after SCT, PD-L1 and PD-L2 levels for diagnosed patients did differ from those of patients who experienced persistence after CTx (P=0.038 and P=0.023).

Summary/Conclusions: Our study shows that HIV status has no prognostic impact on AL patient’s outcome. HIV patient with acute leukemia should thus be included in clinical trials to improve and standardize their therapeutic management.

E911

TEN-DAY DECITABINE AS INDUCTION THERAPY FOR OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA FIT FOR INTENSIVE CHEMOTHERAPY

C. Zhou1, H. Wei1, D. Lin1, Y. Wang1, B. Liu2, K. Liu1, B. Gong3, Y. Li4, G. Zhang1, S. Qiu1, R. Gu1, S. Wei1, X. Gong1, Y. Mi1, J. Wang1.19

Summary/Conclusions: Our study shows that the PD-1/PD-L1 pathway may constitute an immune-escape mechanism in AML. PD-1 expression in CD4+ T cells increased with time since diagnosis. Patients who underwent SCT exhibited overexpression of PD-1, which suggests that SCT and/or chronic stimulation of leukemic cells might induce more PD-1 expression by T cells. Blockade of the PD-1 immune checkpoint may represent an immunotherapeutic strategy for patients with AML relapse or AML persistence after SCT.
with SOC at the end of induction, after completion of the 1st cycle of consolidation, in pts with AML who receive indoximod in combination with 7+3 remission induction consisting of cytarabine (100mg/m²/day for 7 days) and idarubicin (12mg/m²/day for 3 days) and high-dose cytarabine (HDAC) consolidation, as SOC, in pts with newly diagnosed AML (NCT02835729), with a 3+3 design for the phase 1 portion. Indoximod is given orally every 8 hours starting on day 8 of induction onward. Indoximod is recommended phase 2 dose (RP2D) of indoximod in combination with standard of care (SOC) chemotherapy for frontline AML treatment. A key secondary objective is to determine the minimal (or measurable) residual disease (MRD) status, as measured by multi-parametric flow cytometry, in pts with AML who receive indoximod in combination with 7+3 remission induction consisting of cytarabine (100mg/m²/day for 7 days) and idarubicin (12mg/m²/day for 3 days) and high-dose cytarabine (HDAC) consolidation, as SOC, in pts with newly diagnosed AML (NCT02835729), with a 3+3 design for the phase 1 portion. Indoximod is given orally every 8 hours starting on day 8 of induction onward. Indoximod is held on days that pts receive HDAC consolidation, and it is discontinued 4 weeks post-induction, after completion of the 1st cycle of consolidation, and for 4 weeks of consolidation. Eight pts in dose levels 0 and 1 achieved complete remission (CR) after induction. All 8 pts demonstrated no evidence of MRD at levels ≤0.02% (MRD-neg) post-induction and remained MRD-neg post cycle 1 of HDAC. One pt in dose level 0 with favorable risk (normal karyotype, mutations in DNMT3A/FLT3/ITD) and 3 had adverse risk. No RLT was observed with the 1st and 2nd dose levels. The most frequently reported adverse events (regardless of attribution), were febrile neutropenia, diarrhea, nausea and vomiting, dyspnea, hypotension, and hypoxia. Three pts are no longer on study: 1 (dose level 0) due to inability to swallow indoximod after hypoxic respiratory failure during induction, 1 (dose level 1) withdrew consent for personal reasons after only 2 doses of indoximod, and 1 (dose level 2) was taken off due to eligibility. The remaining 9 pts are still on study; 3 pts in dose level 2 are currently receiving induction and are not evaluable. Five of 8 (63%) evaluable pts had dose levels 0 and 1 achieved complete remission (CR) after induction. All 5 pts demonstrated no evidence of MRD at levels ≤0.02% (MRD-neg) post-induction and achieved morphologic CR but had MRD at time of induction, and ultimately relapsed after 2 cycles of HDAC consolidation.

Summary/Conclusions: Indoximod does not appear to add significant toxicity to standard remission induction and consolidation in pts with newly diagnosed AML. Initial data suggest a high rate of MRD-neg after one cycle of induction chemotherapy.

**E913**

**PHASE III STUDY OF MEK INHIBITOR (MEK-162; BINOMETINIB) IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MYELOID MALIGNANCIES**

K. Naqvi1, T. Kadia1, G. Borthakur1, K. Takahashi1, P. Bose1, N. Daver1, Y. Alvarado1, M. Ohanian1, C. DiNardo1, E. Jabbour1, G. Garcia-Manero1, H. Kantarjian1, A. Patel1, F. Ravandi1

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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/RAF/MAPK pathway has shown antiproliferative effects in acute myeloid leukemia (AML) in vitro and in AML patients. 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 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. 11/16 (69%) patients were RAS mutant. 9/16 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 (19%). No dose limiting toxicity was reported.

Summary/Conclusions: MEK-162 shows a tolerable safety profile with an ORR of 10%. The study is currently on-going. Additional studies involving combination of MEK-162 with RAF and PI3 kinase inhibitors are ongoing.

**E914**

**HAPLOIDENTICAL TRANSPLANTATION IS SAFE AND EFFECTIVE FOR OLDER PATIENTS WITH AML/MDS**

S. Clurea1,2, R. Saliba1, M. Shah1, S. Gaballa2, G. Rondon1, J. Chen1, A. Gilbus1, W. Wallis1, B. Oran1, A. Alousi1, Q. Bashir1, S. Ahmed1, D. Mann1, K. Rezvani1, R. Champlin1, K. Chaudhari1, H. Kazahbash1, U. Popat1, I. Khouri1, C. Hosing1, P. Kebrina1, N. Daver2, M. Konopleva1, N. Pemmaraju1, F. Ravandi2, J. Cortes2, H. Kantarjian2, R. Champlin1

1Stem Cell Transplantation, The University of Texas MD Anderson Cancer Center, Houston, United States, 2Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, United States

Background: Acute myeloid leukemia (AML) is more common in the older population. Haploidentical stem cell transplantation (haploSCT) is a potentially curative...
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.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
<th>Median age (range)</th>
<th>Median follow-up (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>AML</td>
<td>25 (58%)</td>
<td>61 (55-69)</td>
<td>19 (6-49)</td>
</tr>
<tr>
<td>MDS/AML</td>
<td>8 (19%)</td>
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<tr>
<td>MDS</td>
<td>10 (23%)</td>
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<td></td>
</tr>
<tr>
<td>Disease status</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CR1/2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>CR1</td>
<td>25 (58%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR2</td>
<td>18 (42%)</td>
<td></td>
<td></td>
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<tr>
<td>Disease stage</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>CR1</td>
<td>25 (58%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR2</td>
<td>18 (42%)</td>
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<td></td>
</tr>
<tr>
<td>Cytogenetics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>16 (37%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>34 (54%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good</td>
<td>11 (26%)</td>
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<tr>
<td>Conditions</td>
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<tr>
<td>HCT-Cl</td>
<td>Median 2 (range 0-11)</td>
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<tr>
<td>Stain source</td>
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</tr>
<tr>
<td>BM</td>
<td>42 (98%)</td>
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</tr>
<tr>
<td>Disease status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR1/2</td>
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<td></td>
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</tr>
<tr>
<td>CR1</td>
<td>25 (58%)</td>
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<td></td>
</tr>
<tr>
<td>CR2</td>
<td>18 (42%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child</td>
<td>33 (88%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibling</td>
<td>2 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor age</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Median 37 (20-65)</td>
<td>28 (65%)</td>
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<tr>
<td>Sex mismatch</td>
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<td></td>
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</tr>
<tr>
<td>Female donor/Male recipient</td>
<td>13 (30%)</td>
<td></td>
<td>30 (70%)</td>
</tr>
</tbody>
</table>

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-Cl was 2 (range 0-11). Reduced melphalan regimen (100mg/m²) was used in 29 pts (67%). Donors were children in 35 (81%) or siblings 10 (19%) patients. Median follow-up was 19 months (range 6-49). One patient died prior to engraftment. Forty-two patients engrafted the donor cells (100%). Median time to neutrophil and platelet engraftment was 19 (13-28) and 28 (15-117) days. Day 30 chimerism was 100% donor in 38 patients (88%). The cumulative incidence of grade 2-4 and 3-4 aGVHD at 6 months post-transplant was 35% and 28% 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 overall survival (OS) and progression-free survival (PFS) was 42%, and relapse rate also in NPM1-mut patients; however, in this cohort NPM1-based MRD integration allows a better risk stratification. MFC MRD is accurate also in NPM1-mut patients; however, in this cohort NPM1-based MRD evaluation is the most accurate predictor of prognosis.

Summary/Conclusions: Our data show that MRD assessment at different time-points during treatment retains a strong prognostic impact in older AML/MDS patients and that different cut-offs at different time-points can give different and useful prognostic information that may drive post-induction therapy. MFC MRD evaluation at TP2 with 0.1% cut-off is the most useful for patients risk stratification. However, the evaluation of MFC-MRD at TP1 with 0.025% cut-off can early identify a group of patients with a significantly low risk of relapse. At the same TP, MFC and WT1 MRD integration allows a better risk stratification. MFC MRD is accurate also in NPM1-mut patients; however, in this cohort NPM1-based MRD evaluation is the most accurate predictor of prognosis.

E916

THE NUMBER OF CD34+CD38+CD117+HLA-DR+CD13+CD33+ CELLS INDICATES POST-CHEMOTHERAPY NEUTROPHIL RECOVERY IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

R. GU1, H. Wei1, Y. Wang1, D. Lin1, B. Liu1, C. Zhou1, K. Liu1, B. Gong1, S. Wei1, G. Zhang1, X. Gong1, Y. Liu1, Y. Li1, X. Zhao1, S. Qiu1, Y. Mu1, J. Wang2.
Background: Hematopoietic recovery is considered to 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 previously data revealed that, CD34+CD38+CD117+HLA-DR+CD13+CD33+ cells (P cells), a kind of progenitor cell, is significantly decreased in patients with delayed neutrophil recovery after chemotherapy compared with that without delayed count recovery.

Aims: To further examine a potential impact of P cells percentage on hematopoietic recovery.

Methods: The data of 223 patients diagnosed with de novo AML was analyzed retrospectively. All these patients enrolled in our previously registered prospective randomized clinical trial AML 2010-01 (201002024). We reviewed the data from bone marrow flowcytometry before the first and second course of consolidation therapy, in which the CD34+CD38+CD117+HLA-DR+CD13+CD33+ progenitor cell percentage in the bone marrow was analyzed. Platelet count recovery time and time of neutrophenia were counted for the evaluation of hematopoietic recovery ability after chemotherapy.

Results: We found that less P cell percentage was significantly associated with prolonged neutrophenia recovery time after the first and second courses of consolidation chemotherapy (p=0.001; p=0.028, respectively). We also observed similar results regard to 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 has demonstrated that P cell percentage was an independent 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 chemotherapeutic hematopoietic reconstitution capacity independently. These findings may help better understand endogenous hematopoietic reconstitution and modify future chemotherapy regimens based on progenitor cell percentages.

E917

MICRONRNAS (miRS) IN HIGH RISK PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) AS PREDICTION TOOLS FOR RCTICTION TIME IN AML. P.P. Leoncini1,*, P. Vitullo1, G. Nigita2, D. Veneziano2, P. Fadda3, V. Tocco1, M. Pigazzi2, G. Basso4, R. Rota1, A Bertaina1

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) (CML) of childhood, the cure rates of high-risk subtypes remain low. Indeed, patients harboring FLT3-ITD mutations or t(12;21) translocations (MLL rearrangements) are still characterized by a poor prognosis, mainly due to leukemia recurrence. Since microRNA (miRs) are small RNA molecules controlling normal hematopoiesis whose deregulation is fundamental in leukemia’s pathogenesis, a possible role as predictors of relapse should be considered.

Aims: Our purpose is to identify, at time of diagnosis, significant miRs signatures able to predict the risk of relapse for patients with high-risk AML, such as FLT3-ITD and MLL rearranged leukemia.

Methods: A total of 204 AML bone marrow (BM) derived samples collected either at diagnosis (ND) and at relapse (RL) together with 8 healthy controls (HCs) were studied (total: N=48). Informed consent has been obtained from either parents or legal guardians according to the Declaration of Helsinki. RNA was extracted, cleaned up and Nanostriing microRNA profiling was performed. Statistical analysis was performed using R software (NanoString Technologies, Seattle, WA, USA) and R-base statistical software. All the assessed results imply a p<0.05 where not mentioned.

Results: Comparing all AML samples with HC, we found 16 up- and 509 down-regulated miRs. Similarly, FLT3-ITD (n=11) vs HCs, showed 17 up- and 361 down- regulated miRs, while MLL-rearranged (n=9) vs HCs showed 16 and 48 miRs respectively. A trend towards down-regulation of the whole cohort was detected and a block in miRs maturation occurring in the 2 molecular subsets was supposed. Finally, a FLT3-ITD vs MLL-rearranged analysis produced a signature in which 20 miRs were up- and 18 down- regulated, a putative signature which could characterize high-risk AML. ND vs HCs analysis identified 17 up- and 297 down-regulated miRs, confirming a tendency toward downregulation, as well as in RL vs HCs analysis, in which we found 12 and 374 up- and down-regulated miRs, respectively. RL vs ND comparison showed a total of 16 up- and 15 down-regulated miRs. In the attempt to identify a signature predictive of recurrence at time of diagnosis, we compared ND and RL samples, revealing 301 miRs that maintained their deregulation in the 2 subgroups, while 113 and 85 were uniquely found in ND vs HCs and RL vs HCs, respectively. Remarkably, miR-34a-5p (P=0.0001) was the recurrent and most statistically significant upregulated miR in both ND and RL samples. Moreover, upregulated miR-10a-Sp and miR-99a-Sp (P<0.0001), and downregulated miR-5p (P<0.0001) were the most statistically significant miRs in FLT3-ITD and MLL-rearranged AML, respectively, underlying putting unique elements distinguishing the two clinical subsets.

Summary/Conclusions: Our results suggest the presence of different microRNA signatures in pediatric AML carrying FLT3-ITD and 11q23 translocations [t(9;11) and t(10;11)]. The identifications of new targets linked to this miRs would pave the way for further studies on the precise molecular basis of this therapy. Interestingly, miR-34a-5p was recurrently found upregulated either in ND and RL groups, but not in the comparative analysis between ND vs RL, suggesting a potential involvement in the mechanisms at the base of both onset and relapse in these subtypes of high-risk AML.

E918

CYTOKINE RECEPTORS AND SOLUBLE ADHESION MOLECULE LEVELS ARE ASSOCIATED WITH PROGNOSIS OF NEWLY DIAGNOSED AML. T. Kupsa1,2, P. Zak2, L. Jevab1,2, J. M. Horacek1,2, *1Department of Military Internal Medicine and Hygiene, Faculty of Military Health Sciences, 24th department of Internal Medicine - Hematology, University Hospital Kralove, Charles University, Faculty of Medicine, Hradec Kralove, Hradec Kralove, Czech Republic

Background: The outcomes of acute myeloid leukemia (AML) treatment are beleaguered by the high recurrence of malignant clones to therapy. Cytokines and adhesion molecules have been studied as markers of immune system activation in many diseases including AML. Furthermore knowledge from baseline cytokine levels assessment may help to improve treatment outcomes.

Aims: The aim of this study is to evaluate baseline levels of selected cytokines, cytokine receptors and adhesion molecules and their relationship with prognosis in newly diagnosed AML patients.

Methods: A total of 75 AML patients, age 52.9±13.0 years, median 58.4 years, 44 female, were studied in the period 2010-2015. Only patients with minimal follow-up of 1 year were included. All patients were induced with “3+7” induction chemotherapy consisting of Cytarabin 100mg/m² 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 IFALda salvage protocols followed by autologous stem cell transplantation in younger and fit patients. In CR, the patients were treated either with HDAIC consolodation and/or allo-hematopoietic cell transplantation or allo-sibling stem cell transplantation in younger patients. A total of 39 patients underwent autologous 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), Erythropoietic Growth Factor, Granulocyte Macrophage Colony Stimulating Factor, Interferon-γ, Macrophage Inflammatory Protein-1α, Monocyte Chemotactic Protein-1, Tumor Necrosis Factor-α (TNF-α), vascular Endothelial Growth Factor, E-selectin (E-Sele, P-Sele), selectin (P-Sele), Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Matrix Metalloproteinase-9, soluble IL-2 receptor-α (sIL-2Rα) and soluble receptors for IL-6 (sIL-6R) and TNF-α type 1 and II receptor-α (sTNF-RI, sTNF-RII) or bihareby labeled as their respective Evidence Investigator analyzer (Randox). Associations of these markers with complete remission (CR) achievement, 1 year progression-free survival (PFS), and 1 year overall survival (OS), were evaluated. Statistical analysis was performed in STATISTICA 2.0.

Results: CR by induction therapy was achieved in 53 cases (70.7%). Those 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-Sele (P=0.0003), higher sIL-2Rα (P=0.0029), higher age (P=0.0356) and possibly with higher TNFR-1 (P=0.0611). Age has not correlated with elevated analyte, TNFR-1 correlates with TNFR-2 (P=0.0196) but not with TNF-α. The sIL-2Rα did not correlate with IL-2. Only IL-6 and ICAM-1 were significantly influenced by CR 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 receptors and adhesion molecules may be markers of immune system activation and could be useful in clinical practice for the prediction of outcome, and thus should be further investigated as possible therapeutic targets. The work was supported by a long-term organisation development plan 1011 (FMHS) and by MH CZ – DRO (UHKK, 00179906).
MRD-DRIVEN CHOICE OF CONSOLIDATION AND MODULATION OF INDUCTION AND CONSOLIDATION INTENSITY RESULTED IN A SIGNIFICANTLY IMPROVED OUTCOME OF YOUNGER AML PATIENTS IN THE LAST THREE YEARS

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Background: In the last decades no effective new drugs have been introduced and AML induction therapy is still based on an anthracycline and cytarabine. The MRC group has, however, reported a progressive increase of cure rates in younger patients. Our group has recently showed that the outcome can be improved by a fludarabine-containing induction (FLAId, with fludarabine administration in first course only), followed by a risk- adapted consolidation.

Aims: The aim of the present study was to evaluate if the disease free survival (DFS) and the overall survival (OS) of younger (<65 years) AML patient treated in our center had shown any modification in four consecutive periods of treatment (< 2008; 2008-2010; 2011-2013; 2014-2016) and to recognize factors possibly leading to this result.

Methods: We reviewed the outcome of 145 consecutive AML patients aged 65 or less and uniformly treated according to the above mentioned strategy. Minimal residual disease (MRD) evaluation was performed by flow cytometry (MFC), assessment of WT1 expression levels and, where applicable, evaluation of recurrent abnormalities such as NPM1 mutation.

Figure 1. Results: The cohorts of patients treated in the four periods had a comparable age and risk distribution. Notably, although the median follow up of the 4 cohorts of patients is different, patients treated in the last 3 years showed a significant improvement in DFS (Fig 1), in comparison with previously treated patients. When we reviewed our experience, we found that some changes we introduced in the therapeutic management, possibly contributed to improve outcome. Beside classical risk factors, the time from hematological recovery after the first induction (induction 1) and the start of the second induction course (induction 2) proved to be significantly related to DFS and OS probability. An interval shorter than 15 days resulted in significantly higher toxicity, whereas a time longer than 28 days was associated with an increased relapse probability. Patients being treated in the last three years had a median time from recovery after induction 1 to start of induction 2 of 17 days, compared to 22 days in the other cohorts (p<0.05). Furthermore, after 2013, MRD information after induction 1 was added as a prognostic factor and ELN low and intermediate risk patient with negative MRD after induction 1 were no more scheduled for early allogeneic stem cell transplant (HSCT), but received an higher dose of Ara-C in each of the three consolidation cycles (12g/sqm cumulative dose vs 8g/sqm). Among 8 intermediate risk patients who were MFC MRD negative post FLAId and did not proceed to HSCT in first complete remission (CR1), only one relapsed whereas among 5 intermediate risk patients who underwent HSCT in CR1 because of MRD positivity no relapses have been observed. Starting from 2014, patient in CR1 not scheduled for HSCT who showed MRD recurrence underwent salvage therapy before overt hematologic relapse, followed by HSCT consolidation. MRD-directed therapy allowed all treated patients to achieve MRD negative remission before HSCT. Finally, the improved outcome may be associated with a reduced incidence of invasive fungal infections (IFI) due to the introduction of prophylaxis with posaconazole. The lower rate of IFI contributed to the reduction in the delay between chemotherapy courses.

Summary/Conclusions: Our experience shows that, even without the contribution of new drugs, more appropriate utilization of HSCT, tailored on early MRD assessment, MRD directed salvage therapy and posaconazole prophylaxis of IFI led to a relevant improvement of outcome.

E920

EFFECTIVENESS OF TREATMENT ACUTE MYELOID LEUKEMIA IN THE ELDERLY USING CLADRIBINE WITH LOW-DOSE ARAC

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1Department of Hematology, Holy Cross Oncology Center of Kielce, Kielce, 2Department of Haematoncology, Medical University of Lublin, Lublin, 3Department of Hematology, Medical University of Lodz, Lodz, 4Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, 5Holy Cross Oncology Center of Kielce, Kielce, Poland

Background: Treatment of acute myeloid leukemia(AML) in the elderly, unfit patients is a challenge for clinical hematologists. Therapeutic management in this patient group is different and numerous complications including early deaths. A standard treatment of low dose cytarabine (LD-AraC) or using hypomethylating therapy is not satisfying enough. Polish Adult Leukemia Group’s (PALG) studies showed, that addition of cladribine to daunorubicine and cytarabine increases the complete remission rate and improves overall survival in younger patients with AML. We also proved effectiveness of cladribine combined with high dose AraC and mitoxantrone in relapsed and refractory AML (1, 2). Cldaridine, 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 one cycle 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, no remission. Median number of cycles to obtain CR was 2 (range 1-3). 16% of patients do not responded to treatment. The regimen was well tolerated without 4-week and 8-week mortality. The main reason of death were: heart failure (n=2), renal failure (n=1) and progressive disease (n=4). We didn’t observe grade 3 and 4. nonhematologic adverse events. With a median time of follow-up 14 months, the median overall survival was 12 months.

Summary/Conclusions: The combination of cladribine plus low dose AraC is effective and well tolerated regimen in elderly AML patients unfit for standard chemotherapy.

E921

SMALL CUSTOMIZABLE NGS BASED TARGET CAPTURE PANELS DETECT VARIANTS IN CLINICAL SPECIMENS AT FREQUENCIES AS LOW AS 0.5%

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Background: The use of large scale hybridization panels in early stages of clinical trials for novel therapies elicits a plethora of information for targeted biomarkers. However, as therapeutic targets are further characterized large panels generate an overly broad set of data, compromising sensitivity in the selected biomarker subset. Therefore, once biomarker targets are identified, the use of smaller hybridization panels can facilitate specific variant detection by analyzing specific genomic regions of interest with greater sensitivity than larger gene panels and PCR-based assays. Modifications of laboratory methods for small scale panels allow for the maintenance of high analytic quality with finely targeted panels. Our small panels (~10kb) are focused on specific genomic regions of interest with greater sensitivity than larger gene panels and PCR-based assays. Modifications of laboratory methods for small scale panels allow for the maintenance of high analytic quality with finely targeted panels. Our small panels (~10kb) are focused on specific genomic regions of interest with greater sensitivity than larger gene panels and PCR-based assays.

Aims: To demonstrate the sensitivity, linearity, concordance with other assays, and clinical applications of small NGS target capture panels.

Methods: Two separate next generation sequencing-target capture assays were developed with bioinformatics software under ISO13485 design control. The panel contained 3 genes, including fms related tyrosine kinase 3 (FLT3); the second covers only CD274 (PD-L1). Libraries were made, hybridized with biotinylated oligonucleotides, and sequenced using the Illumina MiSeqDx. Validation was carried out by spiking in fixed amounts of mutant DNA into wild type DNA to establish the linearity and sensitivity of the assays. Sequencing libraries were generated by conventional methods with kits from both panels. Sequencing data was analyzed using proprietary software developed by Invivoscribe. Eight AML clinical samples were cross validated for FLT3 mutations by this small panel, amplicon based NGS assay, and capillary electrophoresis (CE) assay.
Results: DNA from 24 cell lines was assessed using both panels, confirming variants previously detected using other methods. A validation was run on the 3-gene panel using a series of control samples generated from cell lines containing between 0.5% and 25% variant allele frequencies for expected variants. Initial validation indicates that these small panel assays can detect mutations down to 0.5% variant allele frequencies. Assay linearity for FLT3/TKD detection from 0.25% to 12.5%, or for FLT3/TKD detection from 0.5% to 25% is excellent (R² = 0.996 and 0.998, respectively). Average sequencing coverage was high, ranging from 5,265x to 7,680x. Comparison of FLT3 analysis of the small panel to amplicon based NGS assay and CE, FLT3-ITD showed complete concordance in clinical samples and - showed a strong linear relationship between the targeted VAFs and detected ITD sizes. There was also complete concordance for FLT3/TKD mutations in clinical samples.

Summary/Conclusions: Small hybridization panels are cost effective in detecting low-frequency variants from smaller subsets of genes while using far less DNA than individual PCR-based biomarker assays would require. Additionally, preliminary data shows great accuracy on clinical samples. These smaller assays focus on the most pertinent genes for a targeted therapy, and have the potential to greatly assist in understanding the molecular background of responders, super-responders, and non-responders, information which can help improve patient outcomes. Developing these assays with bioinformatics using the international ISO13485 design control standards makes them suitable for regulatory approval worldwide.

E923

MOLECULAR GENETIC TESTING PATTERNS FOR PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA (AML) ENROLLED IN THE CONNECT® MDS/AML DISEASE REGISTRY


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Background: Recurrent mutations in AML-associated genes have prognostic value and may help guide treatment decisions. Molecular genetic testing patterns for AML in clinical practice are largely unknown. Previous results of the CONNECT MDS/AML Disease Registry (George et al ASH 2016; abstract 354) showed suboptimal adherence to WHO 2008 recommendations for AML diagnosis in a cohort of patients with newly diagnosed AML in clinical practice. Aims: To report a detailed analysis of patterns of molecular genetic testing in patients with newly diagnosed AML in community and academic settings. Methods: The CONNECT MDS/AML Disease Registry (NCT01688801) is a US prospective, observational cohort study of patients with newly diagnosed AML (≥55 years) or myelodysplastic syndrome (MDS). All clinical decisions are made by the treating clinicians. Data are collected, using an electronic data capture system, at screening, enrollment, and approximately quarterly throughout the duration of the patient’s participation in the registry. All patients provided informed consent. Enrollment is ongoing. The current analysis evaluated the percentage of patients with AML who had undergone molecular genetic testing recommended by NCCN guidelines (NPM1, FLT3-ITD, CEBPA, IDH1, IDH2, DNMT3A, and KIT). Chi-square tests evaluated effects of several variables on likelihood of molecular genetic testing. Results: Between 12 Dec 2013 and 8 Dec 2016 (data cutoff), 259 patients with AML were enrolled at 86 sites. Molecular genetic testing was reported in 67% (173/259) of patients. Likelihood of testing varied, respectively, for academic vs community settings (76% [70/92] vs 62% [103/167], P = .018), normal vs abnormal NPM1 (77% [79/102] vs 59% [79/133], P < .005), age <65 vs ≥65 (83% [85/103] vs 50% [108/211]; P = .0003), and before vs after disease diagnosis (81% [83/103] vs 74% [90/122]; P = .025). In patients who had undergone molecular genetic testing (n=173), the mutations tested varied substantially. All of the NCCN-recommended molecular genetic tests were reported in 9% (15/173) of patients. Testing included 8% (6/77) 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 receive guideline-recommended testing. This prospective registry is uniquely positioned to capture changes in testing patterns as guidelines are established.

E924

PHASE 1, OPEN-LABEL, RANDOMIZED STUDY TO EVALUATE THE EFFECT OF CYTOCHROME P450 (CYP) 3A4 INHIBITION ON THE PHARMACOKINETICS (PK) AND SAFETY OF QUIZARTINIB (Q) AND ITS ACTIVE METABOLITE, AC886

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Background: Q is a potent, selective FMS-like tyrosine kinase 3 (FLT3) inhibitor currently being investigated in Phase 3 studies in AML patients (pt) with FLT3 internal tandem duplication (ITD) mutations. Early studies showed concentration (c) and area under curve (AUC) increases of Q and its active metabolite, AC886, was a significant predictor of the QTc prolonging effect(Levis, et al. ASH 2016). Q and AC886 are both primarily metabolized by CYP3A4. Because CYP3A4 inhibiting drugs are frequently required in the...
course of AML treatment, a drug interaction study was performed to assess PK when Q is co-administered with CYP3A4 inhibitors. **Aims:** The primary aim was to determine the effect of ketoconazole (K), a strong CYP3A4 inhibitor, and fluconazole (F), a moderate CYP3A4 inhibitor, on PK of Q and AC886. The secondary aim was to assess the tolerability and safety of Q co-administered with K or F. **Methods:** This was an open-label, randomized, parallel-group study. Healthy subjects (HS) 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 HS were enrolled (31 per arm) and 89 received Q. 75% were male, median age 32 yr (18-53). Relative to Q+P, co-administration of Q+K or Q+F increased the geometric mean (GeoMean) C_{max} of Q by 17% and 11%, and GeoMean AUC_{D1-28} by 94% and 20%, respectively (Table 1 below). The GeoMean C_{max} and AUC_{D1-28} of AC886 were decreased by 60% and 15%, respectively, for Q+K, and were increased by 3% and 14%, respectively, for Q+F. Apparent clearance (CL/F) of Q was 50% lower and t_{1/2} of Q and AC886 were 46% and 96% longer, respectively in Q+K vs Q+P. CL/F of Q was 17% lower and t_{1/2} of Q and AC886 were 10% and 28% longer, respectively, in Q+F vs Q+P. AC886 is a minor component in circulating relative to Q (approximately 25%). An increase of 86% in simulated SS QS C_{max} and 96% in SS QS AUC_{D1-28} was predicted following repeat daily dosing of 30mg Q+K vs Q+F, while a modest decrease in AC886 exposure (<20%) was predicted. The most common (≥5%) adverse events were headache (7.5%) and diarrhea (5.4%), with the majority being Grade 1/2. There were no clinically significant hematology, clinical chemistry, QTc, or vital sign observations, and no deaths or serious adverse events.

**Summary/Conclusions:** Co-administration of Q with K or F was well tolerated and safe. Overall, there was an approximate 2-fold increase in Q exposure when Q was co-administered with K, which is considered clinically significant. The increase in Q exposure when Q was co-administered with F was within 20% and is not considered clinically relevant. Given the relationship between Q conc and Qtc prolongation, these results support reducing Q doses by approximately one-half when taken concomitantly with a strong CYP3A4 inhibitor. No dose reduction is needed when Q is co-administered with a moderate or weak CYP3A4 inhibitor. This approach has been implemented in two ongoing Phase 3 trials of Q in FLT3-ITD mutated AML.

Table 1.

E926

**CLINICAL OUTCOMES OF CHILDHOOD ACUTE MEGAKARYOBLASTIC LEUKEMIA: THE CHILDREN CANCER HOSPITAL EGYPT 57375 EXPERIENCE**

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**Background:** Acute megakaryoblastic leukemia is a rare subtype of pediatric AML occurring in both Down and non-Down syndrome patients. Down syndrome patients with M7 subtype have an excellent prognosis while non-Down syndrome patients have poor outcomes. Heterogenous cytogenetic abnormal-

ities have been described with M7 AML and the impact of different prognostic factors on outcomes is yet to be determined.

**Aims:** To evaluate the predictive significance of various cytogentic abnormalities and minimal residual disease (MRD) by flow cytometry after induction I and correlate them with clinical outcomes of patients with acute megakaryoblastic leukemia.

**Methods:** We retrospectively analyzed the data of 80 non-Down syndrome patients diagnosed with M7 AML treated at CCHE between January 2007 through December 2016. Three treatment protocols were used.

**Results:** The median age at diagnosis was 1.7 years (range 0.2-15). The median time to diagnosis was 1 month. The overall (OS), event free survival(EFS) and cumulative incidence of relapse at 2 years were 53.4%, 42.9% and 28.4% respectively. Sixty one patients had abnormal cytogenetic abnormalities including Trisomy 19 (n=20), 13q (n=3), Trisomy 8 (n=12), Complex karyotype (n=28), t(1;22) (n=12), MLL gene rearrangement (n=9), Trisomy 21 (n=24) but none of these had an impact on outcomes. Out of the 80 patients 56 were in complete remission post induction I. Two hundred patients had <0.1% after induction I. In the univariate analysis patients with MRD <0.1% post induction I had a better OS and EFS with a lower cumulative incidence of relapse however these findings did not reach a statistical significance.

**Summary/Conclusions:** Acute megakaryoblastic leukemia in non-Down syndrome patients have poor outcomes irrespective of any cytogenetic abnormalities. Future direction to determining tumor biology based on molecular pathways in this disease is being considered.

E927

**IDENTIFICATION OF RESISTANCE ASSOCIATED CPG METHYLATION CHANGES IN ACUTE MYELOID LEUKEMIA PATIENTS UNDERGOING INDUCTION CHEMOTHERAPY**

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**Background:** Acute myeloid leukemia (AML) patients ineligible for intensive chemotherapy (IC) are considered current standard of care in this population.

**Aims:** To conduct an indirect treatment comparison (ITC) comparing OS for GLAS+LDAC vs AZA and DEC, respectively. Four studies met inclusion criteria: two studies comparing AZA to LDAC: Fenaux 2010; Dombret 2015; one study comparing DEC to LDAC: Kantarjian 2012, and one study comparing GLAS+LDAC to LDAC: Cortes 2016 (Table). Fenaux 2010 study was excluded due to population differences: baseline median bone marrow blasts at 23% in Fenaux vs 49% in Cortes 2016. The remaining AZA and DEC studies were generally comparable in patient baseline characteristics to the GLAS study: age and cytogenetic risk: age 75/73/76 years old, poor cytogenetic risk 34%/37%/39%, in AZA/DEC/ GLAS+LDAC, respectively. In the ITC, with LDAC as the common comparator, GLAS+LDAC compared favorably with indirect HR for OS vs AZA and DEC being 0.51 and 0.56, respectively, (Table 1).

Summary/Conclusions: Using ITC, treatment with GLAS+LDAC showed significantly better OS HR than AZA and DEC in previously untreated AML patients ineligible for treatment with IC. Limitations of current analysis include mixed IC & NIC population for the AZA trial, and mixed comparator arm of both LDAC and BSC for the DEC trial. Analyses using patient-level data matching baseline characteristics across studies may enable more robust ITC.
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-responders. In this analysis we assessed DNA methylation changes in a group of refractory patients with AML treated either with the hypomethylating agent azacytidine followed by intensive chemotherapy or with intensive chemotherapy alone in order to identify the alterations and genes involved.

Aims: The exploration of whole genome methylation changes of azacytidine and chemotherapy treatment in refractory patients with AML guides treatment refinement.

Methods: Patients from the AML-AZA trial of the Study Alliance Leukemia were randomized to receive either azacytidine followed by chemotherapy or chemotherapy alone. Cells were harvested at baseline and 15 days after chemotherapy from 16 of the 105 patients receiving the combination and from four of the 109 patients randomized to receive chemotherapy only. Genome wide DNA methylation was analysed using a 450K Illumina array (Illumina, San Diego, USA). Methylation changes were compared between the two treatment groups, with 153 days for chemotherapy and 143 days for azacytidine plus chemotherapy 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 INSPI1 (p=1e-17, 6.25% of 176 DMRs), KLF13 (p=1e-14, 7.95%), HIC2 (p=1e-11, 5.11%), while those that were hypomethylated were ARID3A (p=1e-15, 2.82% of 213 DMR’s), MYB (p=1e-14, 3.76%) and STAT1 (p=1e-14, 1.88%). The chemotherapy alone group yielded 7181 DMRs, 5752 of which were hypermethylated and 1429 hypomethylated. The genes most commonly hypermethylated in these patients were EHF (ETS) (p=1e-226, 32.79% of 5752), CEBPE (p=1e-90, 10.34%), and Jun-APII (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 SMAD3 (p=1e-17, 1.05%). Median overall survival did not differ between the two treatment groups, with 153 days for chemotherapy and 143 days for azacytidine chemotherapy treatment.

Summary/Conclusions: Methylation changes associated with azacytidine and chemotherapy of refractory patients were particularly found in genes previously associated with cancer and AML. DNA hypermethylation was more common after chemotherapy alone. This finding suggests that DNA hypermethylation of specific loci may be associated with therapy resistance. Hence, the methylation profiles were detected from the most resistant cells. Of note, upon Azacytidine treatment more hypomethylated loci were observed. This potentially indicates DNA hypomethylation in vivo.
consolidation cycles, the median days to neutrophil recovery was 26 days (range 18-72), platelet recovery 32 days (range 17-75), and the ICU admission rate was 12.8% (range 2-10 days). 18 patients (62.1%) receiving IDAC+2 consolidation suffered disease relapse. For patients receiving IDAC+2 consolidation the median OS was 727 days (range 113-1614 days) with an EFS of 388 days (range 109-1614 days). For patients aged 60-65 years the remission rate and survival outcomes were similar to those published by Lowenberg et al.

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.

E931 DECITABINE COMBINED WITH HAAG REGIMEN IS AN EFFECTIVE SALVAGE TREATMENT FOR ADVANCED ACUTE MYELOID LEUKEMIA

Aims: To evaluate the clinical efficacy and safety of decitabine (DAC) in combination with HAAG regimen ([hydroxarabinone (HKT), cytarabine (Ara-C), doxorubicin (Acla) and recombinant human granulocyte colony stimulating factor (G-CSF)]) for advanced patients with AML.

Methods: Forty-six patients with advanced AML receiving DAC combined with HAAG chemotherapy in our center from December 2012 to August 2015 were enrolled in this study. Of the 46 patients, 18 were refractory or relapsed AML, and another 18 patients were those who didn’t achieve CR after a course of induction chemotherapy. The therapeutic responses, side effects and long-time survival were retrospectively analyzed.

Results: After a course of treatment, the rate of CR and partial response (PR) was 58.3% (21/36) and 22.2% (8/36) respectively, while the overall response rate (ORR) was 80.6% (29/36) in the cohort. For the patients with refractory or relapse AML, CR was 61.0% (11/18), PR was 22.2% (4/18), and ORR was 83.3% (15/18). While for the other not getting CR after a course of induction chemotherapy, CR was 55.6% (10/18), PR was 22.2% (4/18), and ORR was 77.8% (14/18). Grade 4 hematological toxicities were observed in all patients, and 72.2% cases experienced infection. And all non hematological side effects were mild and well-tolerated. With a median follow-up of 7.5 (0.5-33.3) months, the 1-year overall survival (OS) rate was 43.3%, 24.2% for the refractory or relapsed AML patients, and 61.6% for those not achieving CR after a course of induction chemotherapy. The difference was significantly (P<0.01).

Summary/Conclusions: DAC combined with HAAG regimen is safe and effective salvage treatment for advanced stage AML patients.
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 (>2056) were younger, more likely to be married, male, to be working, and to have a higher education than patients living alone. Comorbidity, white blood cell count, lactate dehydrogenase, and blast counts did not differ between groups, however patients living with someone tended to have better performance status at time of diagnosis. Patients living with someone were more likely to receive intensive chemotherapy than patients living alone when aged 60 years or older (41.2% vs 22.8%, adjusted OR 0.81 (CI=0.46-0.81)). In patients <60 years, never-married patients were less likely to receive intensive therapy (adjusted OR 0.43 (CI=0.19-0.99)) than married patients. In patients <70 years achieving CR, the chance of allogHCT was reduced when living alone (11.8%, adjusted OR 0.47 (CI=0.28-0.78), versus 19.0% in patients living with someone). In both groups, 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 AML outcome, especially in patients ≥60 years, is explained by social support rather than by differences in income and occupation. Patients living alone do not present with more advanced disease or higher comorbidity burden than patients living with someone. Still, patients living alone and never-married patients are less likely to receive intensive chemotherapy affected 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 NOTED NPM1 REDUCES TOXICITY OF SALVAGE TREATMENT AND IMPROVES DISEASE CLEARANCE
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Background: Acute Myeloid Leukemia with mutated NPM1 (NPM-AML) is characterized by a favorable prognosis. Most patients achieve hematological complete remission (CR) and are not considered eligible for an early allogeneic stem cell transplantation (SCT). The importance of minimal residual disease (MRD) monitoring in NPM-AML is well recognized but no data are currently available on MRD-directed therapy in this AML subset. Since 2004 we have prospectively evaluated NPM1 MRD at precise time points to evaluate response to therapy and predict the risk of hematological relapse (HR).

Aims: The aim of this study was to set a standardized operating definition of molecular relapse and to evaluate the efficacy and feasibility of MRD-directed salvage therapy.

Methods: From January 2004 to January 2014, 36 consecutive younger intensively treated patients with NPM-AML achieving CR were included in the study. MRD assessment was performed on bone marrow (BM) samples after 1st and 2nd induction cycle, after each of the three consolidation cycles and then every three months for five years. If MRD positivity was found, a new analysis was was 15 days. NPM1 mutation was measured on BM samples using MutaQuant® kit (Ipsogen® from Qiagen. All Real-Time PCR were performed on DN Analyser Option 3.3. Until 2014 our policy included the treatment in hematological relapse (HR). Salvage chemotherapy consisted in two MEC cycles and then patients proceeded to HSCT, if feasible. From January 2015 we decided to use a pre-emptive strategy, treating the molecular relapse. Four consecutive NPM-AML patients who showed MRD relapse received MRD-directed salvage therapy (MEC) which consisted of one cycle of MEC. Five more pts and MRD positivity was detected in 3 pts (0.033%, 0.523 and 3.9%) with intermediate cytogenetic risk. By the end of 4th course 11 pts stayed in CR and we diagnosed 1 morphological relapse (patient with MRD-negativity and CMR after 2nd ChT). Two early relapses were also traced: both with persistent MRD during all period of CR and CMR after the second ChT. All pts with MRD-negative status after first course are alive and in CMR (8 months from diagnosis). While monitoring, LAIP changes were distinguished in 7 pts. One from two with resistant AML lost CD65, another one acquired CD11b. 5 pts were in CR at 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
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Background: Minimal residual disease (MRD) detection by multicolor flow cytometry (MFC) in acute myeloid leukemia (AML) is widely explored by different researchers and it is an additional independent factor in clinical outcomes. The prognostic value of leukemia associated immunophenotype (LAIP) changes in not explored enough.

Aims: To investigate the amount and clearance of MRD reduction and LAIP changes in de novo AML during chemotherapy and compare the results with clinical outcomes.

Methods: In the clinical prospective study since March 2016 till February 2017 50 patients (pts) of de novo AML (median age 44 (17-85)) were included. 14 pts by this moment completed basic chemotherapy (ChT) courses: <7+3 2 induction and 2 consolidation. Among them favorable cytogenetics was in 4pts (t(16,21)-1, 16q22-1, t(8,21)-2pts), intermediate-7 (6-with normal karyotype, 1-t(17,22)), poor-3 (complex karyotype-2, 11q23-1pt). Bone marrow samples were studied in standardized panel with most common antibodies by 6-color MFC (BD FACSCanto II, USA) before the treatment, after 1st and 2nd courses of induction and after 2nd consolidation. Any amount of MRD >0 was assumed as MRD positivity. Besides MRD status we also explored LAIP changes in patients with CR after 2nd ChT and drug resistant MRD 5 pts.

Results: Leukemia associated immunophenotype (LAIP) was detected in all monitored patients at the diagnosis. Molecular markers were detected in 28,5% (2pts-with NPM1+FLT3+CEBPA+, 1-with FLT3+, 1-NPM1+). 2 pts had resistant AML after 2 courses (CR). 3 pts out of 7 with complete morphological remission (CMR) after 1st course had MRD positivity (0.03%, 1.61%, 8.3%) and these pts became MRD-negative after 2nd course. CMR was achieved after 2nd course in 5 more pts and MRD positivity was detected in 3 pts (0.033%, 0.523 and 5.39%) with intermediate cytogenetic risk. By the end of 4th course 11 pts stayed in CR and we diagnosed 1 morphological relapse (patient with MRD-negativity and CMR after 2nd ChT). Two early relapses were also traced: both with persistent MRD during all period of CR and CMR after the second ChT. All pts with MRD-negative status after first course are alive and in CMR (8 months from diagnosis). While monitoring, LAIP changes were distinguished in 7 pts. One from two with resistant AML lost CD65, another one acquired CD11b. 5 pts were in CR at 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.

E935
LENALOMIDE MAINTENANCE IN PATIENTS WITH HIGH RISK ACUTE MYELOID LEUKEMIA
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The aim of this study was to set a standardized operative definition of Minimal residual disease (MRD) monitoring in NPM-AML is well recognized but no data are currently available on MRD-directed therapy in this AML subset. Since 2004 we have prospectively evaluated NPM1 MRD at precise time points to evaluate response to therapy and predict the risk of hematological relapse (HR).
Background: New drug combinations and higher intensity therapy have led to significant improvements in complete remission (CR) rates for patients with acute myeloid leukemia (AML). However, relapsed disease remains a major source of failure. With the exception of allogeneic stem cell transplant (SCT), there are few options for post-consolidation maintenance of remission in high-risk patients. NK cells as part of the immune microenvironment are important mediators of immune surveillance in AML. Lenalidomide has demonstrated single-agent activity in AML and enhances NK cell activity and immune synapse formation in leukemia.

Aims: We designed a phase II clinical trial studying the efficacy of lenalidomide as maintenance therapy in AML patients with high-risk disease in remission, who were not being considered for SCT.

Methods: AML patients ≥18 years with a high-risk feature in 1st CR (CR1) or any patient in 2nd CR (CR2) who had received induction and at least 1 consolidation cycle were eligible for enrollment. Patients should be within 12 months of achieving CR, have PS ≤3, adequate kidney/liver function, ANC >0.5 and platelets ≥30. Patients were treated continuously with lenalidomide 10mg PO daily on D1-28 of a 28 day cycle up to 24 cycles. All pts had baseline cytogenetic and molecular testing, and minimal residual disease (MRD) assessment by flow cytometry. After cycle 1, stepwise dose escalations were allowed to 20mg daily in pts who were tolerating their dose and have presence of minimal residual or morphologically detectable disease.

Results: A total of 14 patients have been enrolled with a median age of 57.5 years (range, 23-67). All pts were in CR at the time of enrollment, with 12 pts (86%) in CR1 and 2 (14%) in CR2. Baseline pt characteristics are outlined in Table 1. AML-related mutations detected at start of therapy include: CEBPA (n=5), NPM1 (3), FLT3 (3), IDH2 (2), NRAS (2), DNMT3a (2), and 1 each of JAK2, TET2, and EZH2. High risk features at the time of enrollment were as follows (some are overlapping): S (36%) with history of prior myeloid neoplasm or therapy related AML, 4 (29%) persistent MRD, 4 (29%) adverse mutational profile, 2 (14%) adverse karyotype, 1 (7%) primary refractory disease, and 2 (14%) CR2 status. Patients have received a median of 9 cycles (1-24) cycles of therapy. With a median followup of 19+ months (8.5-39), the 6- and 12-month estimated RFS were 100% and 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-heme toxicities were 1 each of rash, fatigue, cough, and nausea, vomiting, and stroke.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.5 (35-72)</td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>4.6 (2.5-9)</td>
</tr>
<tr>
<td>Plt (10^12/L)</td>
<td>119 (73-213)</td>
</tr>
<tr>
<td>LDH</td>
<td>599 (209-1129)</td>
</tr>
<tr>
<td>Albinin</td>
<td>4.2 (3.3-4.7)</td>
</tr>
<tr>
<td>Creatinin</td>
<td>0.6 (0.2-1.1)</td>
</tr>
<tr>
<td>Cr (mg/dl)</td>
<td>0.8 (0.4-3.2)</td>
</tr>
</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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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 HAD without HCT in patients ≤60 years. Competing risk cumulative incidence estimates were calculated for NRM and relapse. Early relapse and NRM were defined as relapse/NRM before completion of postremission therapy.

Results: Median age was 52 (18-74) years. Median follow up time was 481 (31-3364) days. Early relapse rate (RR) and NRM were 11.01% and 5.29%, respectively. Median OS after early relapse was only 128 days. Presence of FLT3/ITD mutation and high body mass index were associated with increased risk of early relapse on multivariate analysis (HR 14.88, 95%CI 3.24-68.43 and 2.34, 95%CI 1.3-4.2, respectively). Age increased risk of early NRM (HR 5.13, 95%CI 1.5-17.58 for age 55-35 years). 76% of patients completed therapy: 42% received allogeneic HCT in CR1, 21% completed three cycles of HIDAC and 13% completed two cycles of IDAC. 3-year OS and RFS of the whole cohort were 53.68% and 40.26%, respectively. OS was 67% in a group of patients who completed HCT, 34% in IDAC group and 64% in HCT group (p=0.28469). Cumulative incidence of NRM and RR 3 years after completion of therapy was 23% and 20% after HCT, 7.13% and 51% after HIDAC and 16.8% and 66.4% after IDAC, respectively, differences among groups were significant (p=0.00947 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 1.06-4.43). 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 1.88-19.2), 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

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Background: More and more data on patients over the age of 60 years treated with intensive chemotherapy are emerging, however, long term data with patient outcomes after the initial 2-5 years are lacking. In 2007, we published a single center study on patients over the age of 60 years, suffering from acute myeloid leukaemia (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 of follow up.

Aims: To characterize the longterm outcome of elderly AML and high risk MDS patients treated with intensive chemotherapy after the usual 2-5 year follow up period.

Methods: We treated 160 patients aged 60 years or more suffering from high risk MDS and AML with intensive chemotherapy regimen between 1991 and 2004. None of the patients underwent allogeneic stem cell transplantation afterwards. We now perform 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 leukaemia. 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.

E939

A MULTICENTER, RETROSPECTIVE ANALYSIS OF ELDERLY PATIENTS WITH ACUTE MYELOID LEUKAEMIA WHO WERE TREATED WITH DECITABINE

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Background: While acute myeloid leukaemia (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 arisen in elderly patients.

Methods: Patients diagnosed with AML from 2013 to 2016 were included in the analysis. The inclusion criteria were as follows: (1) 65 or older patients with newly diagnosed, histologically confirmed AML (myeloid blast ≥20% either in the bone marrow or peripheral blood); (2) Treated with decitabine in a schedule of 20mg/m2 for five days every 4 weeks in patients. The primary end-point of the study was OS. We compared our data to the data from another Korean retrospective analysis, in which elderly patients with AML who were treated with decitabine in order to confirm whether the clinical outcomes of this agent are also acceptable in this population, and to provide further understanding of the disease nature of AML arisen in elderly patients.

Results: A total of 80 patients were eligible for the analysis. The median age of patients was 74 years (range, 64 to 86 years) and 49 patients (61.3%) were male. Regarding the risk group, 6 (7.5%), 49 (61.2%), and 25 (31.3%) cases were classified as favorable, intermediate, and poor risk group, respectively. The patients had received median 3 (range 1-27) cycles of treatment and the median OS for all patients was 10.2 months. The median OS durations according to the 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. Next, we compared our data to another Korean retrospective analysis dealing with elderly AML patients who were treated with either best supportive care or intensive treatment. Although our analysis shows that more and more patients with poor performance status and elderly patients, it seems that outcomes of decitabine treatment are fairly better than that of best supportive care (OS 3 months) and comparable to intensive chemotherapy (12.1 months).
DRUG-DRUG INTERACTION POTENTIAL OF GILBERTINIB IN HEALTHY SUBJECTS AND PATIENTS WITH RELAPSED/REFRACTORY ACUTE MYELOID LEUKEMIA


Aims: To evaluate drug-drug interaction potential with gilteritinib in healthy subjects and patients with relapsed/refractory (R/R) AML.

Methods: The effects of CYP3A4 inhibitors (itraconazole [ITZ] and fluconazole [FLZ]), as well as a CYP3A4 inducer (rifampin [RIF]), on the gilteritinib pharmacokinetic (PK) profile were assessed in an open-label, parallel-group study conducted in 81 healthy subjects. Gilteritinib was administered as a single dose alone on Day 6, or in combination with 200mg ITZ administered twice daily on Day 1 and once daily on Days 2–28, or in combination with once daily 400mg FLZ on Day 1 and 200mg FLZ on Days 2–28. When given concomitantly with ITZ or FLZ, gilteritinib was administered on Day 6. In an additional cohort, RIF 600mg was administered on Days 1–21 and gilteritinib was administered as a single 20-mg dose on Day 8. Additionally, the potential inhibitory effects of gilteritinib on the PK profile of a CYP3A4 substrate (midazolam) was assessed in a cohort of patients with R/R AML (n=9) in the Phase 1/2 CHRYSALIS study (NCT02014458). Patients received oral gilteritinib (300mg/d) and single oral midazolam (2mg) doses. Gilteritinib was administered on Cycle 1 Day 1 and continued once daily in 28-day cycles; midazolam was administered on Day-1 and Cycle 1 Day 15. Furthermore, in patients with R/R AML, gilteritinib trough concentration data for patients on strong (eg, voriconazole or posaconazole) or moderate (eg, FLZ) CYP3A4 inhibitors were compared with those for patients not using CYP3A4 inhibitors.

Results: In healthy subjects, gilteritinib exposure (expressed as Cmax and AUC24) was higher (2.2-fold increase) in subjects who were coadministered gilteritinib with a strong CYP3A4 inhibitor (ITZ) than in subjects who were administered gilteritinib alone. Coadministration of gilteritinib with RIF, a strong CYP3A4 inducer, resulted in an approximate 70% decrease in gilteritinib exposure in healthy adult subjects (Figure 1). In patients with R/R AML, midazolam exposure was approximately 10% higher when administered with gilteritinib compared to midazolam alone as reflected by the geometric mean ratio and 90% confidence intervals of midazolam Cmax (111.64%; 69.54%–179.25%) and AUC24 (109.46%; 49.82%–240.48%). Additionally, a <2-fold increase in gilteritinib exposure was observed in patients who were taking concomitant medications that were moderate or strong CYP3A4 inhibitors relative to patients who did not use a CYP3A4 inhibitor. The increased exposure in these patients, however, did not translate to differences in the incidence of drug-related safety events when compared across groups.

Summary/Conclusions: These data suggest limiting concomitant use of strong CYP3A4 inhibitors with gilteritinib. Furthermore, these data suggest coadministration of CYP3A substrates with gilteritinib is unrestricted. A comprehensive review of safety data in patients with R/R AML did not suggest that dose adjustment is warranted when gilteritinib is coadministered with strong CYP3A inhibitors. Although concomitant use of gilteritinib with strong CYP3A inhibitors (eg, ITZ or FLZ) may be permissible, precaution is warranted.

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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

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Background: For decades no effective new drugs or better anthracyclin cytara-bin combinations other than the standard 3 + 7 regimen have been available for AML induction treatment. Fludarabine-based regimens have shown good efficacy in relapsed patients but raised concern about toxicity in the induction setting. Several regimens other than the standard 3 + 7 regimen have shown better tolerance and good results in patients younger than 60 years (ys) (Guolo AJH 2016, PMID 27084986).

Aims: We report a single center, real life experience of unselected 136 consecutive AML patients treated since 2002 in our center with Fludarabine, Aracytin, Idarubicin plus or without Etoposide: FLAIE up to 65 ys or FLAI up to 75ys.

Methods: Patients were treated with the FLAIE or FLAI regimen followed by Idarubicin plus Aracytin as 2 step induction. Exclusion criteria for treatment were: acute promyelocytic leukemia, poor performance status and severe comorbidity. Post remission treatment included up to three cycles of high dose AraC and autologous (Auto) or allogeneic (Allo) stem cell transplantation according to cytogenetic and molecular risk stratification (CMR, Döhner Blood 2010 PMID 19880497) aiming for a curative strategy for all our AML patients.

Results: Median age at diagnosis was 55ys (18-75ys), median follow up was 18 months (range 3–172 months), 75% of patients (102/136) had de novo AML, with 25% (34/136) had secondary AML mostly from myelodysplastic 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 pubi-
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 60ys 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 the tratamiento was well tolerated. However, survival for low SOX4 expression and high SOX4 expression was 26 months 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 had significantly lower karyotype profile and NPM1/FLT3-ITD status compared to patients with intermediate-risk cytogenetics, (P=0,001 and P=0,005 respectively), or even in those with normal karyotype profile (P=0,022 and P=0,111, respectively). In multivariate analysis, high SOX4 expression was found to be an independent poor prognostic factor of OS (RR 2.091, 95% CI 1.051-4.168, p=0.034) 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.

**Summary/Conclusions:** In the current study, we found that AML patients with low BM SOX4 expression had higher remission rates and longer overall survival than those with high SOX4 expression, regardless of age, WBC count at diagnosis, karyotype profile and NPM1/FLT3-ITD status. Our results also reveal that SOX4 is an independent prognostic factor of AML. In conclusion, we reveal that BM SOX4 expression could serve as an informative new biomarker for the clinical prognosis of AML patients.

**E943**

**OVEREXPRESSION OF SOX4 CORRELATED WITH POOR PROGNOSIS OF ACUTE MYELOID LEUKAEMIA**

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**Background:** The SOX4 belongs to the SOX (Sry-related high-mobility group box) family and has been characterized as a transcription factor. Over the past decade, multiple functions of SOX4 have been unveiled, and the protein is now known to play important roles in embryonic development, cell fate decision, and cellular differentiation. Overexpression and amplification of SOX4 have been implicated in various cancers and are correlated with poor prognosis. In mouse models, previous studies demonstrated that the upregulation of SOX4 can be induced by and then cooperate with the aberrant expression of AML-1-ETO, NUP98-DDX10, and PML-RARα; the overexpression of HOXA9, CREB, and Evi1, and the haplosufficiency of PU.1 to trigger leukemogenesis. Furthermore, a previous study that employed retroviral transduction of SOX4 and bone marrow transplantation techniques revealed that increased SOX4 expression may cooperate with the derepression of Mef2c expression to induce myeloid leukemia in recipient mice. Sox4 gene was also reported to be as a direct target of C/EBPα. C/EBPα is known to inhibit the self-renewal of leukemic cells and to restore cellular differentiation. The overexpression of SOX4 that results from C/EBPα inactivation contributes to the development of a type of leukemia that is characterized by a distinct leukemia-initiating cell (LIC) phenotype. This 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 clinicalopathological parameters of de novo AML and to evaluate the prognostic value of SOX4 expression for AML patients.

**Methods:** From March 2009 to December 2011, a total number of 112 adult AML patients were enrolled in this study. This study was approved by the Institutional Review Board (IRB) of the National Taiwan University Hospital (NTUH) and written informed consent was obtained from all participants in accordance with the Declaration of Helsinki. Immunocytochemical staining was used to assess SOX4 expression in bone marrow leukemic cells. All statistical analyses performed for this study involved two-tailed Student’s t-tests, Mann-Whitney U tests and Pearson’s chi-square test or Fisher’s exact test and multivariate analysis with Cox proportional hazards regression models. Kaplan-Meier estimation techniques were used to plot survival curves and log-rank tests.

**Results:** We divided AML patients into two groups according to the intensity and extent of SOX4 expression as follows: low expression group (score 0-2, n=49) and high expression group (score 3-5, n=50), respectively. The various 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 OS (RR 1.924, 95% CI 1.020-3.628, P=0.043) and LFS (19.6 months vs 30.6 months, P<0.001) compared to low SOX4 expression (19.6 months vs 30.6 months, P<0.001). Furthermore, with a median follow-up period of 46.7 months (range: 0.7 to 79.0 months), SOX4 expression was associated with overall survival (OS) and disease-free survival (DFS) in all patients with de novo AML (P=0.008 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 of OS (RR 2.091, 95% CI 1.051-4.168, p=0.034) 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.
mal and minimal platelet count after chemotherapy. 3. rhTPO might shorten the days of platelet count recover to at least 20×10^9/l from its nadir. The incidence of side effects were similar in both groups of the study.

Table 1.

| Table 1. Platelet Parameters of Study Group Compared with Control Group |
|-----------------------------|-----------------------------|
| Control group | Study group | p value |
| Mean number of platelets | 28.3 | 29.1 | 0.154 |
| Mean days of platelet count | 5.2 | 7.1 | 0.0001 |
| Mean daily platelet count | 8.2 | 7.8 | 0.65 |
| Mean number of platelets | 28.3 | 29.1 | 0.154 |
| Mean days of platelet count | 5.2 | 7.1 | 0.0001 |
| Mean daily platelet count | 8.2 | 7.8 | 0.65 |

Summary/Conclusions: rhTPO, administered as dose of 1500U/day when platelet count less than or equal to 50×10^9/l, might improve the recovery of thrombocytopenia of patients with acute myeloid leukemia in CR after consolidation chemotherapy. While there was no significant difference between study group and control group, there was a decreasing trend of platelet transference 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 OS among older AML patients or subgroups thereof, and conducted in the US, EU 5 (United Kingdom, Germany, France, Spain, Italy), or Japan. Only studies enrolling ≥50 patients were included.

Results: Twelve studies (in 19 publications) reporting on OS among older AML patients were included. Participants in most studies were newly diagnosed with AML; ages ranged from 60 to 93 years. Five non-comparative studies examining the effects of various treatment modalities were identified. Median OS in studies examining azacitidine (AZA) ranged from 10 to 12 months, whereas in studies examining induction chemotherapy or reduced intensity conditioning-hematopoietic stem cell transplantation, the median OS ranged from 6.85 to 10.1 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), respectively. Two studies assessed the efficacy of LD-AraC relative to IC, hypomethylating agents (HMA), and BSC. Patients appeared to have longer OS when receiving IC compared to LD-AraC (median OS: 12.4 vs 9.6 months; 3-year OS: 27% vs 12%; p=0.07), and those receiving LD-AraC showed a trend of improved OS (median: 11.5 vs 9.6 months; p=0.001). In one study, same results were obtained as 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. Where multiple HSUVs were available, priority was made for collected values (HSUVs) so that the time AML patients spend in different health states can be aggregated into quality-adjusted life-years (QALY).

Summary/Conclusions: Among older AML patients, IC tended to be associated with improved OS compared with other CCRs. However, evidence from this review indicates that AZA could be an alternative treatment option for older AML patients, whether fit or unfit for IC.
Summary/Conclusions: This interim analysis of the use of decitabine in real life showed a superimposable OS to controlled international clinical trials. Safety profile was acceptable considering setting of pts and incidence of important comorbidities. Despite a similar OS, the comparison between our data and Cashen study (56 vs 55 pts) showed in our cohort, a poorer rate of CR+CRi (60% and 71%), but a superimposable impact of 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 CHRYSANTHemiM 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 (Gin) depletion demonstrates anti-leukemic activity in preclinical studies of AML. We hypothesized that administration of asparaginase Erwinia chrysanthemi (Erwinaze) would lead to effective plasma Gin reduction and may be a feasible therapeutic approach for AML, because myeloblasts may be addicted to Gin.

Aims: The primary aim was to determine the dose of Erwinaze inducing plasma Gin levels ≤120μmol/L, with an acceptable safety profile, 48 hours (h) after the first intravenous (IV) dose and before each subsequent dose administered thrice weekly for 2 weeks in patients (pts) with relapsed or refractory (R/R) AML.

Methods: This was a phase 1, single-arm, pharmacokinetic investigator-initiated trial (NCT02283190, funded by Jazz Pharmaceuticals), with a 3+3 design with dose de-escalation/escalation rules that incorporate both safety and biochemical activity (naïd plasma Gin levels) of Erwinaze. There was no intrapatient dose adjustment. For safety, a 3rd cohort of three pts was to be added if 2 of 6 pts in the 1st and 2nd cohorts experience a dose limiting toxicity (DLT) at a certain dose level. If ≤3 of 9 pts experienced DLT, the trial was to be terminated. To evaluate Gin reduction activity of Erwinaze, the dose could be increased based on 48h trough plasma Gin in cohorts of 3, 6, or 9 pts per dose level. Correlative studies measured plasma Gin, glutamate (Glu) and asparagine (Asn) levels, plasma asparaginase activity and plasma and urine 2-hydroxyglutarate (2-HG) levels.

Results: Five pts were enrolled on study. Enrollment was then halted due to Erwinaze supply manufacturing complexities. Median age was 69 (range 20-83) years, 4 were male, 2 had prior MDS or CMMML, 3 had high risk abnormal karyotype, 3 had isocitrato 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 Gin, Asn and peak Glu levels (μmol/L) at 27 were 28 (range <12.5-227), 0 (range 0-0), and 704 (range 474-754), respectively. Asn remained undetectable for the entire 2 weeks. Gin 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 Gin value <120 μmol/L. The fold reduction (FR) in Gin level at 3 days, relative to baseline, was 0.16 (p=0.031 for rejecting FR=1). One pt achieved partial remission (PR) and one achieved hematologic improvement (HI) after 6 doses of single agent Erwinaze. Both pts had plasma Gin levels <85 μmol/L on days 5, 10 and 12. Off study, after completion of Erwinaze, they have been treated with azacitidine.

Both pts are still alive in complete remission (CR) and CR with incomplete count recovery (CRi) 13.3 and 13.4 months after the on-study date. Plasma and urine 2-HG levels did not change significantly. The 3 pts with IDH mutations tended to have higher plasma 2-HG levels (p=0.10).
Summary/Conclusions: To the best of our knowledge, this is the first clinical report demonstrating that an asparaginase product is capable of not only decreasing plasma Gln level to ≤120μmol/L but also depleting it to undetectable (i.e. <12.5μmol/L) levels in pts with AML. Two of 5 patients with R/R AML had clinical responses and are alive in remission. Given clinical activity of asparaginase in AML, we are to investigate mechanistically-designed asparaginase combination therapies.

E948

PROGNOSTIC SIGNIFICANCE OF SOX2, SOX3, SOX11, SOX14 AND SOX18 GENE EXPRESSION IN DE NOVO ACUTE MYELOID LEUKEMIA (AML) PATIENTS

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Background: Members of the SOX (SRY-related high mobility group (HMG) box) gene family encode a group of transcriptional factors with important functions in embryonic development. Also, SOX genes are aberrantly expressed in different types of cancer. However, their role in hematological malignancies, especially in acute myeloid leukemia (AML), remains elusive.

Aims: The aim of this study was to investigate the expression level of SOX2, SOX3, SOX11, SOX14 and SOX18 genes in de novo AML patients, and to evaluate their potential as prognostic markers.

Methods: Fresh bone marrow (BM) samples were collected from 50 non-APL AML patients at diagnosis (27 male, 23 female, median age 52.5 years, range 22-73) and from 8 healthy donors. Relative quantification analysis of SOX genes expression level was performed by RQ-PCR methodology, with GAPDH gene as endogenous control, and using comparative ΔΔCt method with healthy controls as calibrator.

Results: The median expression level of SOX2, SOX3, SOX11, SOX14 and SOX18 in AML patients was 0.46 (0.01-226.13), 0.81 (0.01-1210.00), 0.35 (0.01-177.29), 0.98 (0.02-469.51) and 3.53 (0.18-332.00), respectively. This was not significantly different from the levels detected in healthy controls where the median expression levels were 1.00 (0.32-2.54), 1.00 (0.45-5.73), 1.00 (0.19-2.83), 1.04 (0.38-2.38) and 1.00 (0.48-12.29), respectively. As a cut-off value above which the patients were considered to be positive for SOX2/3/11/14/18 gene expression we used median expression level of each SOX gene in healthy controls + 2SD. The percentage of patients who were positive for the expression of the studied genes ranged from 14% (SOX2* and SOX11*), 20% (SOX3* and SOX18*) to 28% (SOX14*). A significant association with the presence of FLT3-ITD and NPM1 mutations was detected in all but SOX14+ patients. The same result was found concerning association with higher leukocyte count. There were no significant associations with any other presenting clinical parameters. As for the impact that SOX expression positive status had on the prognosis of the studied genes had on the prognosis and outcome of the disease, we detected higher relapse rate in SOX14+ patients (p=0.045). Significantly shorter disease-free-survival (DFS) was detected among SOX2+, SOX11+ and SOX18+ patients (p<0.001; p=0.001; p=0.017, respectively). Although all of the SOX+ patients had shorter overall survival (OS) time compared to 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 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. Therefore, 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 are candidates for hematopoietic stem cell transplantation (HSCT), an intensive intervention usually reserved for fit patients, even transient decrease in cardiac function might render them ineligible for this intervention, or might increase their transplant related morbidity.

Aims: To study the short-term outcomes of anthracycline exposure on cardiac function in patients with AML who are candidates for allogeneic HSCT. Because current AML-induction regimens use anthracyclines (most commonly daunorubicin) at a relatively high dose between 45 and 90mg/m2/day for three consecutive days, we hypothesized that the incidence of post-induction cardiac injury in patients with AML might be high.

Methods: The medical records of 55 consecutive patients who had received induction chemotherapy and had undergone HSCT in our medical center were reviewed. Patients included in the study were those with echocardiographic data both prior to and post induction therapy. Median age at diagnosis was 59 years (range: 19-73) and 49% were males. Approximately half of the patients had de novo AML (N=29, 53%). 26 patients (47%) had either therapy related AML or AML secondary to a previous hematological disorder. Induction treatment included 7 days of cytarabine at a dose of 100mg/m2/day and 3 days of daunorubicin at a dose of 45mg/m2/day (N=2, 3.6%), 60mg/m2/day (N=34, 61.8%) and 90mg/m2/day (N=15, 27.3%).

Results: Selected patient characteristics are summarized in Table1. Post-induction echocardiogram studies demonstrated a significant cardiac deterioration in left ventricular ejection fraction (EF) (defined as 10% or more absolute decrease from baseline EF) in 25.5% of the patients (N=14). Higher doses (90mg/m2/day) of anthracyclines were associated with a higher incidence of cardiac function deterioration (odds ratio: 4.1, 95% confidence interval: 1.06 to 15.7). Patients with cardiovascular risk factors and male patients tended to develop cardiotoxicity at higher rates, whereas age, white blood cell counts at diagnosis and AML type (de novo vs. secondary) had no impact on cardiotoxicity. The decrease in cardiac function was temporary in 10.9% of the patients (N=6) with subsequent normalization of left ventricular EF in those patients.

Summary/Conclusions: The use of daunorubicin at a dose of 60mg/m2/day or less is associated with significantly lower rates of acute cardiotoxicity. Our findings should be taken into consideration when choosing the anthracycline dose, particularly in male patients with cardiovascular risk factors who are candidates for HSCT.

Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>de novo AML</th>
<th>secondary AML</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>50 (19-73)</td>
<td>57 (18-73)</td>
<td>0.13</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>22 (44%)</td>
<td>26 (54%)</td>
<td>0.36</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>26 (53%)</td>
<td>16 (44%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>40 (27-256)</td>
<td>50 (20-700)</td>
<td>0.04</td>
</tr>
<tr>
<td>Relapse rate</td>
<td>47 (85%)</td>
<td>38 (73%)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression level in AML patients (mean, range)</th>
<th>Expression level in controls (mean, range)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOX2</td>
<td>0.46 (0.01-226.13)</td>
<td>1.00 (0.32-2.54)</td>
<td>0.001</td>
</tr>
<tr>
<td>SOX3</td>
<td>0.81 (0.01-1210.00)</td>
<td>1.00 (0.45-5.73)</td>
<td>0.001</td>
</tr>
<tr>
<td>SOX11</td>
<td>0.35 (0.01-177.29)</td>
<td>1.00 (0.19-2.83)</td>
<td>0.001</td>
</tr>
<tr>
<td>SOX14</td>
<td>0.98 (0.02-469.51)</td>
<td>1.00 (0.48-12.29)</td>
<td>0.001</td>
</tr>
<tr>
<td>SOX18</td>
<td>3.53 (0.18-332.00)</td>
<td>1.00 (0.48-12.29)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
AN INTEGER WEIGHTED GENOMIC MUTATION SCORING (IWGMS) USING THE TRUSIGHT MYELOID SEQUENCING PANEL SHOWS HIGHER MOR-
TALITY IN PATIENTS WITH INTERMEDIATE RISK ACUTE MYELOID LEUKAEMIA: A RETROSPECTIVE STUDY
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Background: AML is currently classified by European LeukemiaNet into favorable, unfavorable, and intermediate prognosis based on cytogenetic aberrations. Although favorable and unfavorable categories have good prognostic values, the intermediate category encompasses the majority of patients and offers unclear prognosis. The development of Cancer Genome Atlas (TCGA) opens new windows for the incorporation of next generation sequencing (NGS) into cytogenetics to enhance prognostic risk stratification. However, few studies explore the combination of cytogenetics and NGS in prognostic predictions.

Aims: Here we have developed a system of Integer Weights for the Genomic Mutation Score (IWGMS) for a quantifiable stratification of the prognostic risks associated with a combination of cytogenetic aberrations and genomic mutations. Our next step is validating the scoring system through its application to data obtained from other institutions.

Methods: Patient data at Houston Methodist Hospital was queried from Methodist Environment for Translational Enhancement and Outcomes Research (METEOR), a clinical data warehouse that integrates research databases and national registries. The diagnosis of AML was queried along with patient demographics, cytogenetics, NGS and OS. The resultant patients were divided into three categories based on their MRC cytogenetic risks: favorable, intermediate, and poor. Using the TruSight Myeloid Sequencing Panel (Illumina), mutations in 54 genes associated with myeloid disorders were tested in NGS. A score was developed that xenografted each of the nine TCGA mutation categories (Transcription- Factor Fusion, 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 intermediate cytogenetic and low mortality subgroups based on a combination of cytogenetic and genetic mutations. We expect similar results with data from other institutions.

Figure 1.

Summary/Conclusions: Most studies in current literature focuses on the individual contributions of cytogenetic aberrations or genetic mutations to risk stratification and treatments risk stratification and treatment response. However, prognosis varies widely in the heterogeneous, intermediate cytogenetic class, where 60% of the AML patients belongs. We propose a systematic approach that correlates cytogenetics with genetic mutations in stratifying prognostic outcomes with a focus on the intermediate cytogenetics group. The ability to differentiate in this specific group opens great potentials for targeted therapies and improving outcomes.

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 lymphocytes of healthy volunteers were also examined. Finally, the effectiveness of amino acid restriction was evaluated in vivo by xenotransplantation of ATL cells into NOG mice. Mice were fed with different diets lacking specific amino acids at 6 weeks after transplantation, and sacrificed at 10 weeks for analysis of peripheral blood, organs, and lymphoma size.

Results: In vitro studies revealed that ATL cells have dependency on specific amino acids: cysteine, methionine, and valine. As 2-weeks restriction of the former two amino acids damaged stromal cells or normal lymphocytes, valine was picked up for further analysis. Proliferation of ATL cells was dramatically inhibited by valine restriction while the influence on normal cells was limited. Interestingly, valine restriction did not effect a significant change in the proportion of normal CD4+ populations, such as Treg, naive, central memory, effector, 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.
**Aims:** Since the roles of these SNPs in clinical aspects, response to therapy and prognosis of DLBCL treated with R-CHOP- are still unknown, these were the aims of the present study.

**Methods:** Our analysis included 168 consecutive DLBCL patients at diagnosis seen at University Hospital from July 2009 to September 2014. Genotypes were identified in DNA of peripheral blood by real-time polymerase chain reaction using a TaqMan SNP Genotyping Assay. Replicates were performed in 10% of the reactions, achieving 100% of concordance. Chi-Square test, Fish-er’s Exact test, and multivariate analysis, using the logistic regression model, served to assess associations between genotypes and clinical aspects. Kaplan-Meier analysis was used to evaluate the effect of clinical features and genotypes on the estimated probability of event free survival (EFS) and overall survival (OS). EFS and OS were calculated from the date of diagnosis to first event (relapse, progression or death by disease) or last seen date and death by any cause or last seen date, respectively. The Cox proportional hazardmodel was used to evaluate the effects of clinical features and genotypes of the above mentioned SNPs on PFS and OS, and the results of analysis were presented as hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). First, these associations were examined using univariate Cox proportional hazards regression. In a second step, all variables with P<0.10 were included in a multivariate Cox regression. All reported P values were two-sided, and P<0.05 was considered to indicate statistical significance.

**Results:** Concerning clinical features, the frequency of the wild-types VEGF-1154G and VEGFR-2-604TT genotype were more common in stage III or IV patients. The wild-type VEGF-1154G allele was more common in high intermediate and high international prognostic index (IPI) patients. Concerning response rate, patients with the wild-type VEGF-936CC genotype was associated with higher complete response (CR). These patients had 2.65 more chances of achieving CR to therapy than others. The median follow-up time of 168 DLBCL patients enrolled in the study was 43 months (range: 1-105). The estimated probabilities of 60-months EFS and OS were 58.8% and 66.0%, respectively. At 60 months of follow-up, patients with the variant VEGF-1154A and 936 T alleles had 1.52 and 1.82 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 G/A SNP was associated with PFS and OS: patients with the variant VEGF-1154 A allele had 1.88 and 1.83 more chances of having an event.

**Summary/Conclusions:** Our data present, for the first time, preliminary evidence that inherited abnormalities in AG pathway, related to the VEGF-1154G, -634GG genotype were more common in stage III or IV patients. The wild-type VEGF-936CC allele was associated with higher complete response (CR). These patients had 2.65 more chances of achieving CR to therapy than others. The median follow-up time of 168 DLBCL patients enrolled in the study was 43 months (range: 1-105). The estimated probabilities of 60-months EFS and OS were 58.8% and 66.0%, respectively. At 60 months of follow-up, patients with the variant VEGF-1154A and 936 T alleles had 1.52 and 1.82 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 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.

**Background:** Interest in the role of myeloid-lineage cells, including monocytes and their precursors, has been increasing in prognosis of lymphoma. It has been shown that the circulating monocyte count at the time of diagnosis shows prognostic significance in diffuse large B-cell lymphoma (DLBCL), suggesting the role of specific subset of monocyte in prognosis of DLBCL. Recent studies suggest CD11b+ monocytes expressing CX3CR1 promote angiogenesis and suppress anti-tumor immunity through the interaction with fractalkine (CX3CL1), the only ligand for CX3CR1. However, limited data is available regarding the prognostic significance of CD11b+CX3CR1+ monocytes in DLBCL patients.

**Aims:** The study investigates the prognostic significance of peripheral blood (PB)- and bone marrow (BM)-CD11b+CX3CR1+ monocytes on progression-free survival (PFS) and overall survival (OS) in newly diagnosed DLBCL patients treated with R-CHOP, and provided informed consents. Percentages of CD11b+CX3CR1+ cells in total mononuclear cells (>50,000 cells) were measured by flow cytometric analysis using fresh PB and BM aspirates taken before treatment.

**Results:** Eighty-nine patients (male, 52) were enrolled. The median age was 65 years (range, 19-88). 37 patients (41.6%) were classified as high-intermediate (HI) or high risk according to National Comprehensive Cancer Network International Prognostic Index (NCCN-IPI). CD11b+CX3CR1+ monocytes were measured in BM and PB samples. A total of 271 DLCBL 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 not treated. Fifty-one patients refused to participate in clinical trials including standard regimens plus new agents (Bortezomib, Lenalidomide, Ibrutinib) or non-standard regimens (R-CHOP/14, Da-EPOCH-R, MACOP-B, Mega-CHOP, Hyper-CVAD). In the homogeneously treated (R-CHOP/21) 205 DLBCL patients subset, the median age at diagnosis was 61 y.o. (range 18-85), with a balanced gender distribution (103 females, 102 males). Twenty-three of these patients (11.3%) had BMI on BM, whereas 43 (21%) had BMI according to PET/CT finding. Fifty-three patients (25.9%) had BMI according to either BM or PET/CT. Concordant BMI by means of both techniques was present in 16 (7.8%) patients. With a median follow-up of 25 months (15-47 months, p25-p75), 50 patients (24.4%) progressed or relapsed and 41 (20%) died. The 3-year estimated progression-free survival (PFS) and overall survival (OS) were 70%, and 78%, respectively. By univariate analysis, factors associated with a shorter PFS, with a p<0.150, were: female gender, IPI>3, abnormally elevated B2-microglobulin levels, PET/CT-BMI(+) and BMI-BMI(+) (in multivariate analysis only two factors contributed to prognostic significance: IPI≥3 and BMI-BMI(+) (p=0.036 and 0.007, respectively). In multivariate analysis only BMI ≥3 (HR: 2.6, 95%CI 1.13-5.14; p=0.006) was independently associated with a shorter PFS. BMI-BMI(+) association, predictive factors of a shorter OS, with a p<0.150, included: IPI>3, abnormally elevated B2-microglobulin levels, PET/CT-BMI(+) (in multivariate analysis only BMI ≥3 (HR: 2.6, 95%CI 1.13-5.14; p=0.006) was independently associated with a shorter OS.

**Summary/Conclusions:** In our DLBCL cohort, treated with a uniform first-line chemotherapy regimen, BMI by BM complemented IPI in predicting those patients with a higher risk for relapse or progression, while IPI defined a subset of patients with a worse survival. In this cohort, BMI by PET/CT could not independently predict a shorter PFS and/or OS.
27.7 months (IQR, 14.6-46.1), low PB-CD11b+CX3CR1⁺ cell group had significantly better PFS (3-year, 77.1% vs 58.7%; P=0.006) and OS (3-year, 86.6% vs 58.4%; P=0.004) than high PB group. No significant survival differences were observed between high and low BM-CD11b+CX3CR1⁺ cell groups. Uni-variates analyses demonstrated that age, ECOG performance status, B symptoms, extranodal involvement, NCCN-IPI, and PB-CD11b+CX3CR1⁺ cell group were significantly associated with OS. However, high risk NCCN-IPI was an only independent prognostic factor for reduced OS (hazard ratio, 4.41; 95% confidence interval, 1.17-15.69) 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 DLBCL cases. In contrast, PB-CD11b+CX3CR1⁺ monocytes failed to predict OS (3-year, 91.7% vs 96.7%; P=0.875) in the low to low-intermediate risk NCCN-IPI subgroup.

Summary/Conclusions: Our study represents PB-CD11b+CX3CR1⁺ monocytes can be used in differentiating patients with high risk for early death and are associated with risk stratification by the NCCN-IPI, possibility of potential therapeutic target in DLBCL.

E955
RARE NON-HODGKIN LYMPHOMAS (R-NHLs) IN CHILDREN: THE AIEOP EXPERIENCE

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Background: Clinical management of pediatric rare non-Hodgkin lymphomas (r-NHLs) (1/1 billion) is unclear.

Aims: To characterize children with r-NHLs in AIEOP centers. Performing a retrospective analysis of r-NHLs AIEOP case records, describing main epidemiologic, clinical and histopathological parameters. To review the histopathological case records according to WHO 2008 classification. Evaluation of treatment response - chemotherapy or wait and see (W&S) in terms of overall survival (OS) and of complete remission (CR), relapse and resistance cases, secondary neoplasias and deaths.

Methods: Data from the AIEOP database were collected between 1997 and 2015. Results: The incidence of r-NHL in AIEOP registry was 6.5% (67 pts). Forty-eight were male and 19 female, median age was 11 years (0.3-21 years). Classification according to St.Jude stage was: stage I n=36; II n=13; III n=11; IV n=7. Bone marrow (BM) involvement was diagnosed in 7 cases; central nervous system (CNS) in one case. Patients who presented LDH >500 UI were 18. B-NHLs accounted for approximately 49% (33 pts) of the entire population analyzed, in 27 cases (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) among B-NHLs; peripheral T-cell lymphoma (PTCL) n.o.s., mycosis fungoides (MF), subcutaneous panniculitis T-cell lymphoma (SPTCL) and lymphomatoid papulosis (LP) amongst T-NHLs; histiocytic sarcoma (HS) amongst “others” category. A similar proportion for both B and T/NK NHL underwent either W&S approach only or active treatment (AT); 45% and 55% were W&S and AT approach, respectively. Patients in “others” category were almost actively treated (71%). Therapy was based on AIEOP B-, T/NK-NHLs and ALCL protocols and +/- conventional RT, and/or immunotherapy. Surgery resection has been performed in case of localized disease B-NHLs only, followed by a W&S strategy, with 3-year 0%-3y 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 frequent event in both B- and T/NK-NHLs, which showed a slight increase of relapses; as for the category “others”, no relative preponderance has been registered for any of the above-mentioned events. 3-year OS has shown to be significantly higher for B-NHLs than for T/NK-NHL (94% vs 69%, p-value 0.024), as illustrated in Figure 1. Regarding the treatment, the 3-year OS was 100% for the patients underwent a W&S approach whereas 75% for treated patients (p-value 0.037). FLs show favourable clinical course and outcome, limited stage at diagnosis. Differently from adults, pFLs have a higher 3-years OS with respect to that of other histological pediatric NHLs subtypes (100% vs 75%, p-value 0.049).

Figure 1.

Summary/Conclusions: The incidence of AIEOP pediatric r-NHLs is in line with the literature. In case of localized disease, a W&S approach was successfully applied; of these, the T/NK NHLs being most often registered and with best prognosis are the cutaneous lymphomas (i.e. LyP, MF). Patients’ prognosis varies greatly depending on the histological subtype. The better survival was observed in the B-NHLs compared to other categories. An international collaboration is warranted, in order to create new guidelines or protocols for an appropriate management of pediatric r-NHLs.

E956
PRIMARY ANALYSIS OF THE EFFECT OF HEMATOPOIETIC STEM CELL TRANSPLANTATION IN THE TREATMENT OF 110 CASES OF T CELL LYMPHOMA

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Background: T cell lymphoma(T-NHL) is a rare and heterogeneous group of lymphoid malignancies with mostly poor outcome with conventional treatment. Recent studies have suggested that Hematopoietic stem cell transplantation(HSCT) has a better curative effect and is superior to traditional chemotherapy.

Aims: To investigate the effect of HSCT in the treatment of T cell lymphoma.

Methods: The clinical data of 110 patients with T cell lymphoma treated by HSCT from January 2006 to August 2016 in our center were retrospectively analyzed.

Results: (1)110 T-NHL patients, 70 males and 40 females, aged 7-64 years (median age 26 years). Disease subtypes: 35 cases of T-cell lymphoblastic lymphoma(T-LBL), 23 cases of NK / T cell lymphoma(NK/TCL), 24 cases of peripheral T-cell lymphoma (PTCL, NOS), 24 cases of variable large cell lymphoma (ALCL), 3 cases of subcutaneous panniculitic T cell lymphoma(SPTCL) and 1 case of hepatosplenic T cell lymphoma(HSTCL). Transplantation type: 56 cases of autologous hematopoietic stem cell transplantation (auto-HSCT), 54 cases of allogeneic hematopoietic stem cell transplantation (allo-HSCT). The follow-up was ended in December 2016, the duration of following-up ranged from 2 to 130 months (median follow-up time was 22 months). (2)35/110 patients with auto-HSCT, 3-year overall survival (OS) and event-free survival (EFS) were 76.5% and 60.9%, respectively. (3)54/110 patients with allo-HSCT, 3 year EFS 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 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).25/54 cases treated with allo-HSCT, the 3 year OS and EFS were 62.8% and 60.8%. 20/56 patients with non-CR1 status before auto-HSCT, the 3 year OS and EFS were 47.8% and 36.9%. The OS and EFS of the two groups were also significantly different (P=0.001).

Summary/Conclusions: HSCT can improve the efficacy of T cell lymphoma. Auto-HSCT in first complete remission (CR1) enables T-NHL patients with
greater benefit. Allo-HSCT can cure some T-NHL patients, which can be considered for the treatment of young and high-risk T-NHL patients.

**E957**

**SHORT COURSE OF R-HYPERCVAD/MITX/ARA-C FOLLOWED BY ASCT AS FIRST-LINE THERAPY IN MANTLE CELL LYMPHOMA PATIENTS PROLONGS PROGRESSION FREE SURVIVAL TO MORE THAN 9 YEARS.**

**SINGLE CENTER EXPERIENCE**

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**Background:** Mantle cell lymphoma (MCL) is considered an incurable disease with an historical median overall survival around 3-4 years with short progression free survival (PFS) periods. Regimens that include high dose cytarabine and consolidation with autologous stem cell transplant (ASCT) have become standard therapy for fit patients. The median PFS reported after 4-6 cycles HyperCVAD followed by ASCT consolidation is 4.5 years (Ahmadi et al, BMT 2012). Nevertheless, toxicity is high and many patients cannot obtain stem cells for transplant. In this setting, some groups use 6-8 cycles R-HyperCVAD without ASCT consolidation, achieving the median PFS of 4.6 years (Romaguera et al, Br J Hematol 2010). Based on this we have review our experience using a short course of HyperCVAD followed by transplant consolidation.

**Aims:** To analyze our experience treating fit patients with MCL in first line with a short course of 2 cycles of R-HyperCVAD followed by consolidation with ASCT.

**Methods:** From January 2002 to August 2016, the patients diagnosed with MCL treated in first line with a short course of 2 cycles of R-HyperCVAD and ASCT were included in this retrospective analysis. International working group response assessment criteria were used, PFS was calculated from the date of start therapy until date of relapse/progression or last contact.

**Results:** During the study period 85 MCL patients were registered: 7 (8.2%) did not receive immediate therapy, 44 (52.4%) were not eligible for intensive therapy due to comorbidities or age and 33 (39.3%) were treated with R-HyperCVAD. Clinical characteristics at diagnosis of these 33 patients were: M/F ratio: 26/7 (78.8%/21.2%), median age: 63 y.o (limits: 40-73), ECOG 0-1: 26 (86.7%), 15 (45.5%) patients had PET/CT findings suspicious for an underlying second malignancy. A total of 33 out of 259 patients with a diagnosis of Hodgkin’s lymphoma and 204 patients a diagnosis of Non-Hodgkin’s lymphoma. Of the 33 patients, 19 underwent further invasive investigation, with a total of 8 patients having a biopsy proven histological diagnosis of a second malignancy (12.7%). Of the 33 patients, 19 underwent further invasive investigation, with a total of 8 patients having a biopsy proven histological diagnosis of a second malignancy (12.7%).

**Summary/Conclusions:** The frequency of incidental malignancies detected by PET/CT imaging in patients with lymphoma was found to be comparable to other similar international retrospective studies. The majority of incidental second malignancies were early stage and gastrointestinal in origin. Further retrospective as well as prospective data may assist in the establishment of guidelines, to address a standardized diagnostic approach to investigating incidental lesions discovered on PET/CT imaging that are suggestive of a second malignancy.

**E958**

**THE FREQUENCY OF INCIDENTAL MALIGNANCIES DETECTED BY PET/CT SCANS IN PATIENTS WITH LYMPHOMA AND THE ASSOCIATED CLINICAL IMPLICATIONS**

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**Background:** PET/CT imaging has a well-established role in the investigation of malignant lymphoma. Given the widespread clinical applications, unexpected findings are occasionally identified. Whilst there is substantial information pertaining to additional primary cancers identified on PET/CT in patients with solid organ malignancy, there is a relative paucity of data in patients with lymphoma.

**Aims:** The primary aim was to identify the frequency of incidental second malignancies detected by PET/CT in patients with lymphoma. Qualitative data related to histological diagnosis and staging, interruptions or obstacles to lymphoma therapy, therapy for the secondary malignancy and the overall impact upon prognosis were also reviewed.

**Methods:** A total of 550 PET/CT images were performed in 298 patients at The Prince of Wales Hospital, Sydney Australia between January 2013 – March 2016. Patients with both Hodgkin’s and Non-Hodgkin’s lymphoma, with PET/CT imaging performed for all medicare-approved indications were included. All PET/CT reports suggestive of an incidental second malignancy prompted further review of electronic medical records, MOSAIC cancer database and paper medical records. Where a clear diagnosis of second malignancy was confirmed, information regarding histological findings and staging, as well as the implications of this diagnosis related to treatment of the underlying lymphoma and impact on overall prognosis was collected.

**Results:** 510 PET/CT scans in 259 patients had confirmed diagnoses of lymphoma. Patients aged 17 to 96 were included in the study, with a median age of 63 years. Of the 259 patients included (M=155; F=104), 55 patients had a diagnosis of Hodgkin’s lymphoma and 204 patients a diagnosis of Non-Hodgkin’s lymphoma. A total of 33 out of 259 patients with a diagnosis of malignant lymphoma had PET/CT findings suspicious for an underlying second malignancy (12.7%). Of the 33 patients, 19 underwent further invasive investigation, with a total of 8 patients having a biopsy proven histological diagnosis of a second malignancy (24.2%). Qualitative information was gathered regarding the patients who did not have further investigation.

**Summary/Conclusions:** The frequency of incidental malignancies detected by PET/CT imaging in patients with lymphoma was found to be comparable to other similar international retrospective studies. The majority of incidental second malignancies were early stage and gastrointestinal in origin. Further retrospective as well as prospective data may assist in the establishment of guidelines, to address a standardized diagnostic approach to investigating incidental lesions discovered on PET/CT imaging that are suggestive of a second malignancy.

**E959**

**CLINICAL IMPACT OF KARYOTYPIC EVOLUTION ON THE PROGNOSIS OF DIFFUSE LARGE B CELL LYMPHOMA**

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**Background:** The acquisitions of additional chromosomal abnormalities are generally accompanied by the emergence of therapeutic resistance and eventually lead to poor treatment outcome in cancers. However, the actual clinical impact of karyotypic evolution on prognosis differs depending on the type of hematologic malignancy. Although several prognostic indexes, including the International Prognostic Index (IPI), revised IPI (R-IPI), National Comprehensive Cancer Network (NCCN)-IPI, and Kyoto Prognostic Index (KPI) which we have developed (Kobayashi T. Blood Cancer J 2016), have the determinants for prognosis, little is known concerning the prognostic impact of karyotypic evolution in diffuse large B cell lymphoma (DLBCL), the most prevalent subtype of non-Hodgkin lymphoma.

**Aims:** We in this study investigated the clinical impact of karyotypic evolution on the treatment outcome of DLBCL.

**Method:** We retrospectively reviewed the medical records of 465 DLBCL patients who were diagnosed and treated with either rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) or with a R-CHOP-like regimen at three independent institutes in Kyoto, Japan, between January 2006 and April 2014. We analyzed the relationship between the number of subclones and prognosis utilizing the Kaplan-Meier curve and Cox proportional hazards regression analysis. We also utilized Fisher’s exact test to investigate the correlation between the number of subclones and the conventional prognostic indexes, i.e. R-IPI, NCCN-IPI, and KPI. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.
and was approved by the institutional review boards of all participating institutes.

Results: Among the 465 DLBCL cases, karyotypic analyses by G-banding were performed on biopsied tumor specimens before the start of treatment in 181 patients. Among the 181 patients, metaphase spreads were available for G-banding in 120 patients. Neither overall survival (OS) nor progression-free survival (PFS) was statistically significantly different between the patients with available metaphase and no available metaphase spreads. Based on the result of G-banding, we next divided the 120 patients with available metaphase spreads into two groups, i.e., patients with karyotypic abnormalities accompanied by ≥2 subclones and patients with 0-1 subclones. We found that the presence of ≥2 subclones was significantly associated with poor OS (3 year OS rates of patients with ≥2 subclones and 0-1 subclones were 67.6% and 82.8%, respectively (p=0.035), and tended to associate with a shorter PFS. Among the 120 patients with available metaphase spreads, the R-IPI-defined high-risk patients and IPI-defined high-risk patients were significantly more frequent in the group of patients with ≥2 subclones. Ages and genders were not significantly different between patients with ≥2 and with 1-2 subclones.

Summary/Conclusions: DLBCL is a cytogenetically and molecularly heterogeneous disease entity. No specific chromosomal abnormality has been associated with the shorter survival, except double or triple hit lymphomas. However, in this study, it was possible to divide DLBCLs into two groups based on karyotypic evolution, i.e., DLBCLs with 0-1 subclones and with ≥2 subclones, because the OS was the most markedly different between these two groups. In our study, more subclones were associated with poor prognosis, suggesting the significance of karyotypic evolution in DLBCL. In conclusion, our study suggests that more advanced cytogenetic clonal evolution underlies the development of high-risk disease feature in DLBCL.

E960

REGIEM 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 COODOX/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 low intermediate IPI risk) and higher risk group (high-intermediate and high). Descriptive statistics were used for baseline characteristics. Kaplan Meier method was used to estimate PFS and OS, and the log-rank test was used to compare OS and PFS between lower and higher risk groups.

Results: A total of 83 patients were included. The M:F ratio was 9:4. Median ages were 45 years (y) (range 25 – 65). Two thirds of patients were Caucasian. The median time from HIV to NHL diagnosis was 29 months (range 0 – 284). Eighty two percent presented with stage III/IV disease. Bulky disease was present in 27%, elevated LDH in 66%, and CD4 count<100/ml at diagnosis in 22% patients. Fifty percent of patients were on HAART therapy at time of lymphoma diagnosis (PFS) was statistically significantly different between the patients with available metaphase and no available metaphase spreads. Based on the result of G-banding, we next divided the 120 patients with available metaphase spreads into two groups, i.e., patients with karyotypic abnormalities accompanied by ≥2 subclones and patients with 0-1 subclones. We found that the presence of ≥2 subclones was significantly associated with poor OS (3 year OS rates of patients with ≥2 subclones and 0-1 subclones were 67.6% and 82.8%, respectively (p=0.035), and tended to associate with a shorter PFS. Among the 120 patients with available metaphase spreads, the R-IPI-defined high-risk patients and IPI-defined high-risk patients were significantly more frequent in the group of patients with ≥2 subclones. Ages and genders were not significantly different between patients with ≥2 and with 1-2 subclones.

Summary/Conclusions: DLBCL is a cytogenetically and molecularly heterogeneous disease entity. No specific chromosomal abnormality has been associated with the shorter survival, except double or triple hit lymphomas. However, in this study, it was possible to divide DLBCLs into two groups based on karyotypic evolution, i.e., DLBCLs with 0-1 subclones and with ≥2 subclones, because the OS was the most markedly different between these two groups. In our study, more subclones were associated with poor prognosis, suggesting the significance of karyotypic evolution in DLBCL. In conclusion, our study suggests that more advanced cytogenetic clonal evolution underlies the development of high-risk disease feature in DLBCL.
patients with tumor localized in extranasal sites seemed to have higher expression of BCL2 than higher DHS than nasal lesions (p=0.014 and 0.042, respectively). In univariate survival analysis, either high expression of MYC or BCL2 was significantly correlated with inferior PFS and OS (p<0.05). According to the DHS, patients with ENKTL could be divided into three significantly different risk groups for PFS and OS (3-year PFS rate for DHS of 0, 1, and 2 was 60%, 41%, and 21%, respectively, p=0.006; 3-year OS rate for DHS of 0, 1, and 2 was 79%, 49%, and 33%, respectively, p=0.015). In multivariate survival analysis, it was found that DHS was an independent prognostic factor for both PFS and OS (p=0.006 and 0.011, respectively).

Summary/Conclusions: Our study demonstrated that DHS can help identify patients with newly diagnosed ENKTL who are at a high risk for a poor clinical outcome, which needs to be validated in prospective clinical trials with patients treated uniformly.

E962

SOLUBLE INTERLEUKIN-2 RECEPTOR AS A PREDICTIVE MARKER FOR SPONTANEOUS REGRESSION OF OTHER IATROGENIC IMMUNODEFICIENCY-ASSOCIATED LYMPHOPROLIFERATIVE DISORDERS: A RETROSPECTIVE STUDY

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Background: Patients treated with immunosuppressive drugs (ISD) for autoimmune diseases are at an increased risk of developing other iatrogenic immunodeficiency-associated lymphoproliferative disorders (OI-LPD). Some patients with OI-LPD shows spontaneous regression after withdrawal of ISD, but some require chemotherapy. The factors that are associated with spontaneous regression and outcomes of chemotherapies remain uncertain.

Aims: The aims of our retrospective study are to assess the clinical factors that predict spontaneous regression of lymphoma after ISD withdrawal in patients with OI-LPD and to evaluate the outcomes of patients who underwent chemotherapy without spontaneous regression.

Methods: We collected data from all patients with autoimmune disease who were pathologically diagnosed with OI-LPD between January 2002 to October 2016 at Yokohama City University Hospital, and Yokohama City University Medical Center.

Results:

The patients included 12 males and 28 females, with a median age at diagnosis of 65 years (range 30-81). Methotrexate (MTX) was administered to all patients at any point of the clinical course before OI-LPD. The median time from diagnosis of autoimmune disease to OI-LPD development, and the median duration of MTX administration were 12 months (range 1-89 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, and 2 patients with lymphoma presenting as extranodal lymphoma.

The patients with spontaneous regression after ISD withdrawal in patients with OI-LPD showed sIL-2R levels <2,400 U/mL, and the median duration of MTX administration were 129 months (range 11-564), and the median duration of sIL-2R administration were 65 months (range 3-240). The CR rates, 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.

E963

PROGRAMMED DEATH-1 PROTEIN EXPRESSION AND ITS RELATION WITH HISTOLOGIC AND CLINICAL VARIABLES IN MYCOSIS FUNGOIDES

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Background: Mycosis fungoides (MF) is a T-cell malignancy with affinity for the skin. In early stages, treatment directed to the skin can induce long-lasting remissions. However, advanced stage MF may have short-duration remissions and progressive disease. The programmed death cell surface protein-1 (PD-1) is expressed on activated T cells. Interactions between PD-1 and its ligands control the induction and maintenance of peripheral T-cell tolerance during the normal immune response. These interactions may also play a role in the immune evasion of tumors in which PD-1 ligand is overexpressed.

Aims: To describe histologic characteristics and the proportion and intensity of PD1 expression by tumor cells, as well as the presence of PD1 positive lymphocytes in the epidermis in patients with MF. To identify histologic variables that might have an impact in clinical outcome.

Table 1.

<table>
<thead>
<tr>
<th>Characteristic of Patients</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>20-40</td>
<td>30%</td>
</tr>
<tr>
<td>Stage</td>
<td>IA-IB</td>
<td>40%</td>
</tr>
<tr>
<td>sIL-2R intensity (IU/mL)</td>
<td>&lt;2400</td>
<td>60%</td>
</tr>
<tr>
<td>Tumor cell expressing PD1</td>
<td>Low</td>
<td>60%</td>
</tr>
<tr>
<td>Proportion of tumor cells</td>
<td>10%</td>
<td>60%</td>
</tr>
<tr>
<td>Antitumor/anti-PD1 cells</td>
<td>No</td>
<td>50%</td>
</tr>
<tr>
<td>Proportion of tumor cells</td>
<td>10%</td>
<td>60%</td>
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<tr>
<td>Antitumor/anti-PD1 cells</td>
<td>Yes</td>
<td>50%</td>
</tr>
<tr>
<td>Proportion of tumor cells</td>
<td>10%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Results:

The patients included 39 patients, of whom 20 were males and 19 were females, with a median age of 65 years (range 20-80). The most common site of diagnosis was the skin (87%), followed by the lymph nodes (13%). The median duration of disease was 60 months (range 3-240). The most common site of diagnosis was the skin (87%), followed by the lymph nodes (13%). The median duration of disease was 60 months (range 3-240). The most common site of diagnosis was the skin (87%), followed by the lymph nodes (13%). The median duration of disease was 60 months (range 3-240). The most common site of diagnosis was the skin (87%), followed by the lymph nodes (13%). The median duration of disease was 60 months (range 3-240).
CIRCULATING MICRORNAS AS BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA: A PILOT PROSPECTIVE LONGITUDINAL CLINICAL STUDY

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Background: Diffuse large B-cell lymphoma (DLBCL) is highly heterogeneous in terms of phenotype and treatment response in patients. These characteristics make the prognosis difficult to establish and hinder the use of new personalized treatments in clinical practice. In this context, there is currently a necessity to define new biomarkers enabling a better definition of DLBCL subtypes, prognosis evaluation and an overview of the resistance to chemotherapeutics. We decided here to focus on circulating microRNAs that are found in all biological fluids. 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 B303201419613). Informed consents of all patients were obtained. In this way, blood samples from patients were taken before any treatment (C0), at the administration of the second and the fourth chemotherapeutic cure (C2 and C4) and at the remission review (Cf). In the case of an autograft, a sample was taken at the post-graft review (Cpost). 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.

Conclusion: The purpose was to evaluate the prognostic factors and treatment outcome of patients with diffuse large B-cell lymphoma (DLBCL) of the tonsil. Methods: In all, 114 patients with DLBCL of the tonsil with stage I or stage II, treated at multicenter in Korea, from September 1995 to April 2011, were included. The median age was 59 years and the majority of patients (61%) were male. Symptomatic symptoms were present in 6% of patients. International prognostic index (IPI) score was 0 in 54 patients (48%), 1 in 40 (35%), 2 in 14 (12%), and 3 (3%). Ten patients (8%) showed elevated level of lactate dehydrogenase (LDH). Treatment consisted of a combination of chemotherapy (CTX) and radiotherapy (RTx) for 38 patients (34%) and 72 patients (65%) received CTx only. Among those receiving RTx, the median RTx dose was 39 Gy. Results: After median follow-up of 32 months (range 0.4–106 months), event free survival (EFS) and overall survival (OS) were 25.9% and 42.5%, respectively. Significant prognostic factors included: age ≥60 year-old vs <60 year-old, LDH level (> upper normal limit and supper normal limit), IPI score (0-1 vs 2-3), and treatment (CTX plus RTx vs CTx only). On multivariate analysis: LDH level (hazard ratio [HR], 10.522; 95% confidence interval [CI], 2.548–43.449, p=0.001) and treatment (HR, 12.393; 95% CI 2.151-71.410) were independent prognostic factor of EFS and age (HR, 8.920; 95% CI 1.089-73.053, p=0.043), LDH (HR, 8.316; 95% CI 1.914-36.127, p=0.005), and treatment (HR, 8.943; 95% CI 1.089-73.425) retained statistical significance in OS.

Figure 1.

Summary/Conclusion: LDH level and age significantly influence outcome. A combined modality treatment, consisting of CTx and RTx, results in a satisfactory outcome in patients with stage I or II DLBCL of the tonsil.

E965

COMBINED CHEMOTHERAPY PLUS RADIATION THERAPY IS MORE EFFECTIVE IN LIMITED-STAGE DIFFUSE LARGE B-CELL LYMPHOMA OF THE TONSILS.

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Background: Primary extranodal non-Hodgkin’s lymphomas of the head and neck account for 10-20% of all non-Hodgkin’s lymphomas. Primary tonsillar lymphoma accounts for less than 1% of head and neck malignancies, although the tonsil is the most common primary extranodal site of head and neck non-Hodgkin’s lymphomas.

Aims: The purpose was to evaluate the prognostic factors and treatment outcome of patients with diffuse large B-cell lymphoma (DLBCL) of the tonsil.

Methods: In all, 114 patients with DLBCL of the tonsil with stage I or stage II, treated at multicenter in Korea, from September 1995 to April 2011, were included. The median age was 59 years and the majority of patients (61%) were male. Systemic symptoms were present in 6% of patients. International prognostic index (IPI) score was 0 in 54 patients (48%), 1 in 40 (35%), 2 in 14 (12%), and 3 (3%). Ten patients (8%) showed elevated level of lactate dehydrogenase (LDH). Treatment consisted of a combination of chemotherapy (CTX) and radiotherapy (RTx) for 38 patients (34%) and 72 patients (65%) received CTx only. Among those receiving RTx, the median RTx dose was 39 Gy.

Results: After median follow-up of 32 months (range 0.4–106 months), event free survival (EFS) and overall survival (OS) were 25.9% and 42.5%, respectively. Significant prognostic factors included: age ≥60 year-old vs <60 year-old, LDH level (> upper normal limit and supper normal limit), IPI score (0-1 vs 2-3), and treatment (CTX plus RTx vs CTx only). On multivariate analysis: LDH level (hazard ratio [HR], 10.522; 95% confidence interval [CI], 2.548–43.449, p=0.001) and treatment (HR, 12.393; 95% CI 2.151-71.410) were independent prognostic factor of EFS and age (HR, 8.920; 95% CI 1.089-73.053, p=0.043), LDH (HR, 8.316; 95% CI 1.914-36.127, p=0.005), and treatment (HR, 8.943; 95% CI 1.089-73.425) retained statistical significance in OS.
E968

CLINICAL RELEVANCE OF SARCOPENIA IN DIFFUSE LARGE B-CELL LYMPHOMA - TWO ARE BETTER THAN ONE

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Background: Sarcopenia is known to be associated with poor clinical outcome in patients with diffuse large B-cell lymphoma (DLBCL). There is no consensus concerning the optimal method to define sarcopenia in DLBCL.

Aims: In this study, given the uncertainty about the optimal SMI to define clinically meaningful sarcopenia in DLBCL, we compared the characteristics and clinical outcome between sarcopenic patients determined by L3 skeletal muscle index (L3-SMI) and those determined by pectoralis muscle SMI (PM-SMI) who were treated with R-CHOP21 and more intensively treated patients; there was no difference in age, gender, stage, elevated LDH or PS ≥2. Patients receiving R-CHOP had similar response rates as those receiving more intensive regimens (80% vs 85%, P=0.405), but inferior EFS (HR 2.12, 95% C.I. [1.09-4.12], P=0.028) and OS (HR 2.15, 95% C.I. [1.07-4.3], P=0.034) (Figure 1). 5-year EFS rates were 48% and 76% and 5-year OS rates 80% and 90% for R-CHOP21- and more intensively treated patients, respectively. Differences in outcomes between R-CHOP and intensified regimens remained significant in a multivariate Cox regression model adjusted for age, gender and presence of bulky disease (HR 2.45, 95% C.I. [1.11-5.4], P=0.019 for EFS).

E969

INTENSIFIED TREATMENT REGIMENS IMPROVE EVENT-FREE AND OVERALL SURVIVAL IN YOUNGER NEWLY DIAGNOSED HIGH-RISK PATIENTS WITH B-LARGE CELL LYMPHOMA: A MULTICENTRE OBSERVATIONAL STUDY OF KROHEM

S. Basic-Kinda1,*, M. Lucijanic2, I. Hude1, Z. Prka2, Z. Mitrovic2, I. Radman1, T. Valkovic34, D. Radic-Kristo36, D. Lozic1, D. Nemet8, V. Pejsa29, I. Aurer19, 1Division of Hematology, Department of Internal Medicine, University Hospital Centre Zagreb, 2Division of Hematology, Department of Internal Medicine, University Hospital Dubrava, Zagreb, 3Division of Hematology, Department of Internal Medicine, University Hospital Centre Rijeka, 4Medical School, University of Rijeka, Rijeka, 5Division of Hematology, Department of Internal Medicine, University Hospital Merkur, Zagreb, 6Medical School, University of Osijek, Osijek, 7Division of Hematology, Department of Internal Medicine, University Hospital Centre Split, Split, 8Medical School, University of Zagreb, Croatia

Background: Standard therapy for newly diagnosed B-large cell lymphoma (B-LCL) is R-CHOP. Patients with high-risk disease have unsatisfactory outcome. Non-randomized trials have suggested that intensified regimens, such as R-CHOEP14 and DA-R-EPOCH, improve treatment results in younger patients.

Aims: We performed this analysis to compare response rates, event-free (EFS) and overall survival (OS) rates of newly diagnosed patients with high-risk disease treated with R-CHOEP21 and more intensive regimens (R-CHOEP14 and DA-R-EPOCH).

Methods: Outcomes of B-LCL patients younger than 60 with aaIPI ≥2 treated at two different centres with R-CHOEP14 and DA-R-EPOCH were collected retrospectively from patient files and compared to outcomes of patients with same characteristics treated with R-CHOEP21 from the registry of KroHem, the Croatian Cooperative Group for Hematologic Diseases. All three regimens were administered according to standard guidelines for 6-8 cycles. Patients in PR or with initial bulky disease were irradiated after the end of systemic treatment. Twelve patients treated with DA-R-EPOCH were autografted in 1st remission.

Results: 54 patients were treated with R-CHOEP21, 40 with R-CHOEP14 and 22 with DA-R-EPOCH. R-CHOEP14 and DA-R-EPOCH treated patients did not differ in response rates, EFS and OS and were grouped together for further analysis. R-CHOP treated patients had less frequently bulky disease (25% vs 49%, P=0.007) than more intensively treated patients; there was no difference in age, gender, stage, elevated LDH or PS ≥2. Patients receiving R-CHOP had similar response rates as those receiving more intensive regimens (80% vs 85%, P=0.405), but inferior EFS (HR 2.12, 95% C.I. [1.09-4.12], P=0.028) and OS (HR 2.15, 95% C.I. [1.07-4.3], P=0.034) (Figure 1). 5-year EFS rates were 48% and 76% and 5-year OS rates 80% and 90% for R-CHOP21- and more intensively treated patients, respectively. Differences in outcomes between R-CHOP and intensified regimens remained significant in a multivariate Cox regression model adjusted for age, gender and presence of bulky disease (HR 2.45, 95% C.I. [1.11-5.4], P=0.019 for EFS).

Figure 1.
I. Hude1,*, S. Basic-Kinda1, I. Radman1, S. Dotlic2, M. Kralik3, M. Vodanovic1, (B-LCL) DIAGNOSED ELDERLY PATIENTS WITH B-LARGE CELL LYMPHOMA IN A REGIMEN WITH SIMILAR EFFICACY FOR TREATMENT OF NEWLY SUBSTITUTING DOXORUBICIN WITH ETOPOSIDE IN R-CHOP RESULTS E971

Moreover, comorbidities contribute to inferior survival through frailty, drug CL patients, and can better describe patients within risk groups, compared to Summary/Conclusions:

months

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, respectively.

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), 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%). Poor European Cooperative Oncology Group (ECOG) performance status ≥2 had 145 patients (20.5%). Bone marrow involvement was present in 97 patients (13.7%). At least one comorbid condition had 309 patients (43.6%), while high Charlson Comorbidity Index (CCI) had 44 patients (6.2%). Majority of patients had cardiovascular disorders (223, 31.5%), endocrinological (63, 8.9%), pulmonary (18, 2.5%), psychiatric (13, 1.8%), nephrotic (8, 1.1%), autoimmune (6, 0.8%), and other (13, 1.8%). According to IPI, low, low intermediate, high intermediate and high risk had 332 patients (46.9%), 174 (24.6%), 132 (18.6%), and 70 (9.9%), respectively, while according to NCCN-IPI, 139 (19.6%) patients had low risk, 335 (47.3%) low intermediate, 198 (28.0%) high intermediate, and 70 (9.9%) 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=18.95, 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, and can better describe patients within risk groups, compared to IPI. Moreover, comorbidities contribute to inferior survival through frailty, drug dose reduction and poorer tolerability.

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: 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-CHOP, the standard regimen used at our centre for non-frail elderly without significant cardiac comorbidities. The dose of etoposide in R-CEOP was 50mg/m2 iv or 100mg/m2 orally 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-CHOP. 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-CHOP 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-CHOP group. Efficacy was similar, 65% responded to R-CEOP and 79% to R-CHOP. 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-CHOP group, 3-y EFS was 50% and 50%, respectively (figure). Outcomes of patients with GC and non-GC DLBCL categorized according to Hans’s algorithm were similar irrespective of treatment.

E969

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.

Summary/Conclusions: Long-term outcomes of newly diagnosed B-LCL patients treated with R-CEOP seem as good as those achieved with R-CHOP 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.
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 dual umbilical cord blood and 1 autologous. Of the 52 PTLD, 48 were B lymphomas (92.3%), of which 26 were diffuse large B-cell lymphomas (DLBCL) (54.2%), 7 polymorphic (14.6%), 7 low-grade (14.6%), 4 Burkitt lymphomas (8.3%), 1 Hodgkin’s lymphoma (2.1%) and 1 T/NK non-classifiable. Other 4 PTLD were T lymphomas (6.7%), 2 anaplastic, 1 T/NK lymphoma, and 1 gamma/delta T lymphocytosis. 35/52 PTLD were EBV + (67.3%). The median time of immunosuppression was 123 months in renal transplant, 93 months in liver, 85.5 months in cardiac, 51 months in lung and 3 months in HCT. Histologically, it was 96 months in T lymphomas and 80 months in B lymphomas, being 51 months in EBV + and 124 months in EBV-.

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 virus reactivation or autoimmune diseases. Survival is significantly lower than in other primary LPS. -AR-SA-We analyzed the following data: type of transplantation, immunosuppression used in both induction and maintenance, immunosuppression time from transplantation to lymphoma diagnosis, clinical stage, response to treatment and survival. We classified them morphologically according to the WHO criteria (2016 revision), using immunohistochemical and molecular techniques and their relationship with the Epstein-Barr virus (EBV) by studying the expression of EBERs. We correlated clinical data with morphological, immunohistochemical and molecular results.
Bleeding disorders (congenital and acquired)

E974

AN EXPERIENCE WITH LONG ACTING FACTOR VII PHOPHYLAXIS IN PAEDIATRIC AND YOUNG ADULT PATIENTS WITH HAEOMOPHILIA A

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Background: Hemophilia is an X linked inherited bleeding disorder. Recurrent Joint bleeds and muscle bleeds are the comon 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 dosage schedule is limited.

Aims: To study the efficacy and safety of long acting factor VII (Elocote) 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 thrombophelbitis 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 coagulation disorder resulted from mutations in the FVII gene (F7). The disease severity is not correlated with FVII levels but might be determined by molecular defects in F7.

Aims: To delineate the phenotypic and genotypic characteristics of patients with congenital FVII deficiency.

Methods: We described demographic data, clinical manifestations, and outcome of patients with congenital FVII deficiency. F7 mutation analysis was performed by PCR-direct sequencing.

Results: Of the ten patients diagnosed with FVII deficiency, five (50%) were males. The median age (range) at diagnosis was 19 days old (1-730). Consanguinity was found in 50% of the patients. Of the nine patients (90%) classified as severe, six patients presented with intracerebral hemorrhage within the first month of life, two presented with gastrointestinal bleeding and one presented with hemarthrosis. There were eight different alterations identified. Four have been previously reported (c.1091G>A (p.R364Q), c.1238G>A (p.R413Q), c.1265C>T (p.T419M), and c.681G>T (IVS6+1T)). Four were novel (c.1192C>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.

E976

RETROSPECTIVE EVALUATION OF PHENOTYPE AND MANAGEMENT OF A-HYPO-FIBRINOGENEMIA IN A COHORT OF ITALIAN PATIENTS

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Background: A-hypofibrinogenemia (AF) and hypofibrinogenemia (HF) patients (pts) experience hemorrhages or thromboses, and the clinical management can be difficult.

Aims: To obtain information on AF/HF clinical phenotype and management.

Methods: This is a spontaneous, retrospective, multicenter national study. Data are collected from clinical records.

Results: 2 AF and 12 HF pts have been enrolled (6M, 8F). Median follow-up: 39 months (1-553). Median fibrinogen activity/antigen level: 78mg/dL (0-150)/73mg/dL (0-140). Five pts experienced epistaxis, hematomas, ecchymoses, menometrorrhagia, intra-abdominal bleeding, gum hemorrhagia. Fresh frozen plasma, fibrinogen concentrate (FC), cryoprecipitate, whole blood, tranexamic acid were administered in the majority of these events. One ischemic stroke, 1 lower limb arterial and 1 cerebral sinus thrombosis, 1 concomitant aortic and inferior vena cava thrombosis occurred: 3 events during FC therapy, 1 during puerperium. Heparin, low molecular weight heparin (LMWH), anti-platelet agents, fibrinolytic agents, warfarin were then administered. One gastrectomy, 1 lower limb amputation, 5 gynecological, 1 otorhinolaryngological and 1 plastic surgery were performed in 2AF and 3 HF pts: in AF 5 and 0 respectively, in HF 2 and 3 respectively. One venous thrombosis, 2 hemorrhages, 1 DIC and 4 complicated pregnancies were recorded. FC was administered at delivery and LMWH during puerperium, for the 2 CS. No complications at delivery occurred.

Summary/Conclusions: AF and severe HF pts experience severe hemorrhagic/thrombotic events. The intervening clinical situations are difficult to manage. Further large scale data collections are necessary in order to provide useful information to better characterize and manage patients suffering from these rare diseases.

E977

RETROSPECTIVE REVIEW OF FOUR YEARS OF VON WILLEBRAND’S FACTORS AS SURGICAL PROPHYLAXIS IN VON WILLEBRAND’S DISEASE: S. Bai1, M. K. Ria2, D. Peters3, J. Palascak4, S. Girmas5

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Background: Von Willebrand disease (vWD) is the most common inherited bleeding disorder that manifests as easy bruising, mucocutaneous bleeding and excessive hemorrhage with invasive procedures. In 2007, Humate-P, a lyophilized concentrate of purified VWF and FVIII, was approved in the United States for treatment and prophylaxis. Current guidelines per National Heart, Lung, and Blood Institute (NHLBI) Expert Panel recommend 40-60 U/kg pre-operatively, followed by maintenance dose of 20-40 U/kg for 7-14 days for major surgery and 5 days for minor surgery. Here, we report a single-institution experience of a short course of Humate-P as surgical prophylaxis.

Aims: To assess if an abbreviated schedule of Humate-P given as perioperative dosing of Humate-P 40 U/kg pre for 2 days (one dose pre-op and one post-op) for one pre-op and one post-op for definitive dental procedures and for 4 days (one dose pre-op and 3 days post-op) for minor and major surgical procedures as surgical prophylaxis would result in equivocal efficacy without compromising patient outcomes.

Methods: We retrospectively identified 202 patients with known diagnosis of vWD at our institution that underwent surgical procedures requiring prophylaxis between 2002-2017. Ninety elective surgical events occurred among these patients that required peri-operative prophylaxis with Humate-P. These patients were treated with peri-operative dose of 40 U/kg on D0-1 for extensive dental interventions, D0-4 for D3-5 for minor and D0-3 for D5-7 for major surgery. The definition of bleeding risk was based on NHLBI guidelines.

Results: Eighteen (20%) were males and 72 (80%) were females. Type I vWD constituted 94.4% (85/90). 84.4% Caucasian patients, 8.8% African American, 1.1% South Asian. Twenty-nine (32.2%) procedures were categorized as major operations (36.6%) were minor surgical procedures. Eighty-four (93.3%) achieved excellent hemostatic efficacy defined as clinical hemostasis within normal limits. Six (6.6%) surgeries had good hemostatic efficacy defined as slight oozing. Five (6%) patients required blood transfusions.
Six (38%) patients have 2 mutant alleles and three mutations were recurrently identified. The most frequent mutation detected in this study was Cys389Gly detected in 37% (11/30) patients, validating the data of our previous patient cohort.

**Summary/Conclusions:** Correlation of genetic data with coagulation laboratory and clinical findings suggested the presence of modifiers, which warrants further investigation in a larger cohort of patients for better clinical prediction and management in this rare bleeding disorder.

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**E978**

**AUDIT ON MANAGEMENT OF HIGH INTERNATIONAL NORMALIZED RATIO (INR) IN WARFARINISED INPATIENTS**

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**Background:** Warfarin is the commonest used oral anticoagulant with an effective anticoagulant. The British Committee for Standards in Haematology guidelines recommend administration of 25-50µg of four factor Prothrombin Complex Concentrate (PCC) and intravenous (IV) Vitamin K 5mg for patients with major bleeding, 1-3mg of Vitamin K intravenously for those with minor bleeding and 1-5mg of Vitamin K orally for patients with INR >8 and who have no signs of bleeding.

**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 9/83 (10.8%) non-bleeders with an INR between 5-8. Additionally, the recommended dose and route of administration of Vitamin K 1-5mg PO was followed only in 7/16 (44%) of non-bleeders with INR >8.

**Summary/Conclusions:** Our audit highlighted that there is less than 100% compliance in the recommended dose and route of vitamin K administration. A flowchart containing the guidelines will be designed to improve the management of high INR. To increase the awareness of this issue, teaching sessions for junior doctors and nursing staff are planned. A re-audit will be conducted once these steps are in place.

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**E979**

**NOVEL AND RECURRENT F7 MUTATIONS IN KOREAN PATIENTS WITH COAGULATION FACTOR VII DEFICIENCY**

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**Background:** Coagulation factor VII deficiency is one of rare hereditary bleeding disorders with relatively limited clinical and genetic data. This study aimed to characterize F7 gene mutational patterns of Korean patients with coagulation Factor VII deficiency including their clinical and laboratory variability.

**Methods:** F7 gene mutations of total 16 unrelated Korean patients with Factor VII deficiency were identified by direct sequencing analyses of all exons and flanking intronic sequences. Variants were assigned according to the recently released criteria of 2015 ACMG standards and guidelines.

**Results:** A total of 14 mutations (pathogenic or likely pathogenic) were detected including four novel mutations (Glu66Lys, c.681+3A>T, Glu66Alafs, ile290del).
E980

Bone marrow failure syndromes incl. PNH - Clinical

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, CD157-PE, CD15-PerCP-Cy5.5, CD64-APC, CD45-APC H7 for PNH screening and compared the results with the routinely used 6 color panel. Laboratory cutoff for CD157 was defined by running 10 samples from healthy individuals. Sensitivity analysis was assessed in spiking experiments by diluting a PNH positive sample with normal sample. The results obtained from CD157 were assessed by analysing a total of 30 samples across a wide range of PNH clone size (0.06-97.3%).

Results: CD157 was sensitive at the level of 10^{-4} and better. Frequency of cells with PNH phenotype in normal samples were found to be <0.002%. The CVs of intra-assay precision analysis ranged from 0.92/6.1 to 3.2/1.64% for granulocytes and 1.92/5 to 5.3/6.8% for monocytes. The PNH clone size, as obtained by CD157 based analysis was highly comparable to those obtained by CD24/CD14 based assay (R²>0.993). CD157 was found much better than CD24/CD14 based assay (R2>0.993). CD157 was sensitive at the level of 10^{-4} and better. Frequency 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.

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, hemolytic anemia, β-thalassemia, ITP, hepatitis C, multiple myeloma. In the majority of cases patients with premalignant syndrome of AA (Burkitt's lymphoma, BM of 33 healthy donors were analyzed as normal control group. 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 CD56 (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 (AA) were assessed as “A”, 21.4% (n=3) - “B” and 14.3% (n=2) - “C”. Increased proportion of CD117 and expression of CD56 on CD34+ polymorphs was assessed by analysing a total of 30 samples across a wide range of PNH clone size (0.06-97.3%).

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 CD36 on CD34+ myeloblasts, granulocytes and monocytes is commonly found in AA patients. Perhaps the appearance of MFC dysplastic features foreshadows the MDS-transformation of AA, but requires further prospective studies.

E983

SURGICAL MANAGEMENT OF PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) – DATA FROM THE SPANISH PNH REGISTRY

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Background: Bone marrow failure syndromes include paroxysmal nocturnal hemoglobinuria (PNH), aplastic anemia, Fanconi anemia, and dysmyelopoiesis. The aim of our study was to analyze the surgical management of patients with paroxysmal nocturnal hemoglobinuria (PNH), aplastic anemia, Fanconi anemia, and dysmyelopoiesis in Spain.

Methods: A total of 14 patients with PNH were included in the study, 13 male and 1 female, median age of 59 years. The surgical procedures performed included bone marrow transplantation (BMT), autologous stem cell transplantation (ASCT), and allogeneic stem cell transplantation (AlloSCT). The outcomes of these procedures were evaluated based on the rate of engraftment, the incidence of complications, and the overall survival rate.

Results: All patients received a successful engraftment, with a median time of 21 days. The incidence of complications was low, with only one patient developing graft-versus-host disease (GVHD) and one patient experiencing a bacterial infection. The overall survival rate at 1 year was 78.6%.

Summary/Conclusions: The surgical management of patients with paroxysmal nocturnal hemoglobinuria (PNH) is a complex process that requires a multidisciplinary approach. Our study demonstrates the feasibility and safety of bone marrow transplantation (BMT), autologous stem cell transplantation (ASCT), and allogeneic stem cell transplantation (AlloSCT) in patients with PNH. Further research is needed to improve the outcomes of these procedures and to evaluate the role of new therapeutic strategies in this patient population.

Aims: Here we report data on the clinical management and treatment results of patients with PNH undergoing surgery.

Methods: We collected data on 14 surgical interventions of 11 patients (8 males; age, 25-76 years). All patients had a high prevalence of PNH clone cells (55-99% in PMN) and were receiving eculizumab (ECU). Types of surgery were: 6 laparoscopic cholecystectomies, a transjugular intrahepatic portosystemic shunt, a distal splenorenal shunt, a laparoscopic Achilles allograft ligation, a gastrectomy, an emergency appendectomy, and 3 urologic interventions. Ten patients received ECU 900mg, while one (patient E, surgery 6) received 1200mg since he had developed hemolysis at a previous surgical intervention (surgery 5). In two cases (patient G, surgery 8; patient H surgery 11), an additional dose of ECU was administered before surgery. Patient H (surgery 11) had developed hemolysis at previous surgical interventions (surgeries 9 and 10). In most cases, either the date of the ECU dose was taken into account when scheduling surgery or the ECU dose was moved forward to coincide with the date of surgery. The time between the last ECU dose and surgery was normally one day (range, 1-8).

Results: In nine cases, transfusions were required due to 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.
Summary/Conclusions: Clinical outcomes with eculizumab were significantly improved compared with the baseline in patients with both PNH/AA and classic PNH. This study demonstrated that eculizumab has a beneficial role in the management of patients with PNH/AA, similar to that of classic PNH, by inhibiting hemolysis and reducing transfusion requirements, thus resulting in the improvement of clinical signs and symptoms.

E985
DIAGNOSIS AND FOLLOW-UP OF THE CLONES OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA BY FLOW CYTOMETRY
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a very rare chronic disease associated with a clonal expansion of one or several hematopoietic stem cells carrying acquired somatic mutations of PIG-A gene resulting in GPI-AP deficient blood cells and great susceptibility to complement mediated cell lysis. Diagnosis of PNH is of importance and flow cytometry (FC) is a required tool for this. We report 33 cases of PNH diagnosed and monitored by FC.

Aims: To show the interest of flow cytometry for the diagnosis and follow-up of PNH clones in some risky haemopathies.

Methods: A PNH clone has been researched in 234 patients since August 2009 to January 2017. The PNH clone was investigated for bone marrow aplasia with or without haemolysis, regenerative hematologic 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 mononclonal antibodies: Fear and CD59 with gaging on CD45 for neutrophils, CD59 with gating on CD54 for monocytus and CD59 with gaging on Glycophrin A for red blood cells. We judged that the patient has a PNH clone when the deficiency is >50% on at least two markers highlighted on two different lines. FC surveillance is provided in the absence of a deficit or in the case of a very low 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, hematologic 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 (72.8%) and mixed deficits in 6 cases (19.2%). The mean degree of CD59 deficiency was 29.4% (5-82) on red blood cells 48.21% (5-95) on neutrophil (N); the mean degree of Fear was 55.33% (6-99) on N in 22 cases ; the mean degree of CD14 deficiency on monocytes was 44% (7-97) in 17 cases, the mean degree of Fear (8 cases) was 51.8% (12.9-92). During surveillance, PNH clone appeared in 02 cases and clone size increased in 1 case. Patients with at least two GPI-liked antigens decreased 2,8- and 1,9-fold, respectively, and further in CR this tendency persisted. Duration of IST-free period less than 1 year and ≥1 year was accompanied by a significant and stable decrease of NK-T cells in PB and BM. It is known that PNH-clone presence is a favourable factor for treatment response of AA. Therefore, this study is important to study the behaviour of NK-T cell clone in AA remission with the size of PNH-clone. In subgroup with small PNH-clone (0-1 %) NK-T cell clone was decreased as compared to primary AA patients, and it significantly decreased further along with growth of PNH-clone size.

Table 1. NK-T cell level (%) in patients with AA in remission according to subgroups.

Summary/Conclusions: Thus, in patients with AA the decrease of NK-T cell level was observed along with recovery of hemopoiesis in all the subgroup variants. Previously we have shown that the decrease of NK-T cells accompanies the recovery of any cytopenias and in case of AA, this pattern, taking into consideration our earlier obtained data, may be the evidence of the role of NK-T cells in regulation of balance Th1:Th2 and produced by them cytokines.

E987
A NOVEL DUAL-REAGENT SINGLE TUBE FLOW CYTOMETRIC ASSAY TO SCREEN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA
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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell disorder resulting from loss of membrane-bound glycosyl phosphatidylinositol (GPI) anchor protein. The disease is characterized by heterogeneous clinical phenotypes including intravascular hemolysis, cytopenia(s), bone marrow hypoplasia and atypical site thrombosis. Screening guidelines recommend documentation of the lack of at least two GPI-APs on at least two cell lineages. Alexa fluo 488 conjugated fluorescent Aerolysin (FLAER-AF488) has become a mandatory component in FCM based PNH assays.

Aims: We have analyzed the feasibility of a novel dual-reagent assay for screening a single tube, single line approach for PNH screening.

Methods: EDTA anti-coagulated peripheral blood of patients referred to department of Hematology with clinical suspicion of classical-PNH/ aplastic anaemia, was tested with a single tube panel of FLAER-AF488/CD33/CDAPC. Simultaneously, the routine two tube flow cytometry assay (established sensitivity of 0.1%) was performed for PNH screening. FLAER-AF488 (€80000000CF granulocytes and FLAER/CD33/CD14 for monocytes) was performed in the same sample. Each tube was run till a minimum of 50,000 granulocytes were acquired or till the tube ran dry. A cluster of at least 20 FLAER negative events was considered for reporting PNH-clone positivity in both granulocytes and monocytes. The granulocytes and monocytes 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 positive clones were further confirmed by FLAER-AF488 in granulocytes and monocytes by both strategies, indicating complete concordance at a sensitivity of 0.2% (Chi Square p=0.000). Of the PNH positive cases, the mean PNH clone sizes among the granulocytes by dual-reagent and conven-
E888 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 severe aplastic anemia (AA) refractory to immunosuppression with a simple, cost-effective and widely applicable dual-reagent, single tube PNH-screening assay at a sensitivity of 0.2%. The study needs to recruit patients of various hematological disorders besides healthy controls, and although seems effective for analyzing classic and subclinical PNH, the strategy has to be further standardized to achieve a sensitivity of 0.01%.

Methods: Retrospective analysis of six patients with a diagnosis of aplastic anemia and thrombocytopenia (platelet count ≤30,000/μL), refractory to immunosuppressive therapy and ineligible for allotransplant, treated with 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 danazol (1). Treatment with eltrombopag was associated with significant decreased (1502±1327 vs 2515±1826; p<0.0063) granulocyte PNH clone sizes (Pearson’s r=0.993, p=0.000) and the monocyte PNH clone sizes (Pearson’s r=0.991, p=0.000) detected by both the analysis strategies.

Summary/Conclusions: This pilot study demonstrates the practical feasibility of a simple, cost-effective and widely applicable dual-reagent, single tube PNH-screening assay at a sensitivity of 0.2%. The study needs to recruit patients of various hematological disorders besides healthy controls, and although seems effective for analyzing classic and subclinical PNH, the strategy has to be further standardized to achieve a sensitivity of 0.01%.

E899 DECREASED EXPRESSION OF ADHESION MOLECULES IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS OF PATIENTS TREATED WITH IBRUTINIB
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Background: The B-cell receptor (BCR) pathway in CLL plays a well recognized role in the onset and progression of the disease and the resulting development of mechanism-driven drugs is revolutionizing the therapeutic manage-
ment. Ibrutinib is a BTK inhibitor that plays an important role in the BCR pathway and induces several alterations in CLL cells.

Aims: The modulation of the expression of adhesion molecules on the surface of CLL cells from patients treated with ibrutinib has been evaluated to analyze the effect of treatment on the relationship between the microenvironment, that promotes cell survival and proliferation, and the leukemia cells with the conse-
quent cell mobilization and increased drug exposure.

Methods: In a cohort of 101 CLL patients treated with ibrutinib (420mg/die) and rituximab (375mg/m²/week) in the GIMEMA CLL1114 trial, we evaluated, before and after 15 days of therapy, the surface expression on leukemic cells of several adhesion molecules. In detail, using 8 color antibody combinations (all from Becton Dickinson, BD, San José, CA) we evaluated the MIF expression (using the FACSDianto II, BD) of CD11a, CD18, CD38, CD40, CD43, CD44, CD49d, CD62L, CD69, CD80, CD86, CD154, CD184 on CD5/CD19+ leukemic cells.

Results: The number of CD5/CD19+ did not increase after 15 days of treatment (52.8±58.5 vs 53.4±51.5 x 10^6/L; p=0.36) probably because of the concomitant rituximab administration, which 'masks' the mobilization effect induced by ibrutinib. We observed a significant down-modulation of CD46 (261±435 vs 171±148; p<0.0001), a molecule (L-selectin) that has been reported as the key factor controlling the binding of CLL cells to the endothelial walls in vivo. CD69 expression resulted also significantly decreased (744±784 vs 438±716; p<0.0041), is expressed on CLL cells in the tissue microenvironment, both in the bone marrow and in lymph nodes. We observed the significant down-modulation of the expression of CD43 (3265±2282 vs 2515±1826; p=0.0063) of several adhesion molecules, and therefore do not seem a reliable marker in patients treated with ibrutinib. On the contrary, CD81 expression, another antigen utilized for MRD detection, resulted unchanged after 15 days of treatment. CD185 expression was significantly decreased (1502±1327 vs 804±687; p<0.001), while we unexpectedly observed the up-modulation of CD164 (2244±2022 vs 3162±1877; p=0.003), both antigens participate in the BTK signaling pathway. CD40, that interacts with activated CD4+ T cells, resulted down-modulated (722±467 vs 395±262; p<0.0001). CD38 and CD49d, when expressed in >20% of the leukemic cells, resulted significantly down-modulated (p<0.028 and p<0.021) both molecules have a role in the crosstalk between the leukemia cells and the microenvironment. Despite these changes, the expression of the CD11a, CD18, CD43, CD44, CD80, CD86 and CD154 did not change.
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 pathways 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 lipodabetic CLL cells utilize free fatty acids (FFA).

Aims: (A) Determine whether CLL cells are capable of utilizing FFA for energy production. (B) Determine whether lipid metabolism in CLL is LPL dependent.

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 ibritinib-treated patients. We used an immuno 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 fresh and immortlized CLL cells with FFA. The concentration of cultured media-dissolved O2 (dO2) prior to and after adding FFA, assuming that if the cells oxidize the acid, dO2 levels will drop. Indeed, after 48 hours incubation with FFA dO2 levels were markedly reduced as compared with the dO2 media levels of CLL cell incubated without FFA. Remarkably, unlike cultured normal cells, small levels of cultured CLL B cells did not change dO2. Intriguingly, the levels of dO2 remained unchanged if CLL cells were incubated in the presence of FFA and ibritinib. Similarly, the dO2 levels of CLL cells obtained from ibritinib-treated patients remained constant, suggesting that ibritinib 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 triglycerides to FFAs. Indeed, we detected LPL in the cytoplasm 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, transcription 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 ibritinib.

E992

INHIBITION OF ARGININE UPTAKE VIA HUMAN CATIONIC AMINO ACID TRANSPORTER-1 (CAT-1): A NOVEL APPROACH FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) THERAPY

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Background: Interference with cancer metabolism by specifically restricting the availability of extracellular arginine. Arginine uptake into cells is currently explored clinically in phase I-II studies. An alternative, largely unexplored strategy to restrict nutrient availability for cancer cells would be to target the specialized cell membrane transporter proteins.

Aim: (i) If CLL cells depend on exogenous arginine, (ii) which transporter is responsible for arginine transport in human CLL cells and (iii) if the reduction of arginine availability via knockdown of this transporter inhibits CLL cell growth and viability.

Methods: Experiments were performed with both, primary human CLL cells, isolated from highly leukemic peripheral blood, and immortalized CLL cell lines. Primary CLL cells were left unstimulated or were stimulated via Toll-like receptor 9. The expression levels of arginine transporters were determined by quantitative RT-PCR and Western Blot. Arginine uptake was measured by [3H]-arginine import, cell proliferation by [3H]-thymidine DNA incorporation and cell viability by the MTS assay in flow cytometry. The expression of hCAT-1 was downregulated in HG3 CLL cells using lentiviral siRNA technology. HG3_hCAT-1 knockdown cells were injected s.c. in NOD/SCID/gcnull mice and tumor growth was monitored.

Results: We show that primary and immortalized CLL cell proliferation depends on the availability of extracellular arginine. Screening a large panel of individual CLL patient samples and different immortalized CLL cell lines demonstrated that hCAT-1, y+LAT1 and y+LAT2 are the predominantly expressed arginine transporters. Upon activation the expression level of hCAT-1 further increased significantly. Arginine uptake in both, primary and immortalized HG3 CLL cell lines, was inhibited by the CAT inhibitor N-ethylmaleimide. Lentiviral downregulation of the hCAT-1 transporter in HG3 CLL cells resulted in a significant reduction of arginine uptake, associated with an inhibition of cell proliferation and viability in vitro. The corresponding in vivo data of tumor growth upon hCAT-1 knockdown in a murine xenograft model will be presented at the conference.

Summary/Conclusions: Our results demonstrate that the hCAT-1 transporter is a potential pharmacological target structure in CLL cells. Development of small molecule- or antibody-based inhibitors of hCAT-1 might lead to a novel therapeutic approach for CLL.

E993

FCMR IS A NEGATIVE REGULATOR OF B-CELL RECEPTOR SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: Chronic lymphocytic leukemia (CLL) cells frequently display features of anergic B cells, including reduced B-cell receptor (BCR) signaling capacity and downregulation of membrane IgM (mIgM). These features are particularly evident in freshly isolated peripheral blood (PB) CLL cells belonging to the indolent, M-CLL subset (Lanham S et al, Blood. 2003). The mechanism responsible for this phenomenon is still unclear, but chronic stimulation with autoantigens has been considered as a possible explanation because of the observation that BCR signaling capacity and mIgM expression can spontaneously recover in CLL cells following prolonged in vitro culture (Mockridge CI et al, Blood. 2007). An alternative explanation for this phenomenon is that these anergic features are induced by soluble IgM molecules, which are absent from standard culture media, and could interact in vivo with the leukemic BCRs through recently described intermembrane immunoglobulin interactions or by binding to the Fc receptor (FcR). The latter is highly overexpressed in CLL cells, particularly those belonging to the M-CLL subset (Li FJ et al, Blood, 2011). An implication is the role of Fc receptor interactions in regulating the BCR signaling capacity.

Methods: CLL cells were isolated from PB or lymph nodes (LNs) using standard procedures. FcR stimulation was done using pentameric human Fc fragment, whereas BCR stimulation was done using goat anti-human IgM or anti-human Ig light chain F(ab')2 fragments. FcμR knockdown was done by RNA interference using the Nucleofector system and solution VICTOR-909 program. Surface FcPr and IgM levels were measured by flow cytometry on gated CD19+/CD5+ cells.

Results: We recently reported that FcμR stimulation results in activation of certain downstream BCR signaling pathways and increased CLL cell survival in vitro (Gobessi S et al, ASH 2016, abstract 2015). To investigate whether FcμR regulates BCR signaling capacity, we analyzed activation of downstream signaling molecules in CLL cells that had been pretreated for one hour with Fcμ and then stimulated with an anti-lg light chain antibody. Decreased phosphorylation of SYK, AKT and ERK in FcμR knockdown mice, suggesting that FcμR negatively regulates BCR signaling in CLL cells. Consistent with this finding, we also observed that FcμR knockdown by RNA interference resulted in greater activation of SYK, AKT and ERK in anti-IgM stimulated primary CLL cells. Because IL-4 was recently shown to increase BCR signaling capacity and surface IgM expression on CLL cells (Aguilar-Hernandez MM et al, Blood. 2016; Guo B et al, Blood 2016), we next investigated whether it will have an opposite effect on FcμR expression. Stimulation of CLL cells (n=7) for 48 hours with IL-4 resulted in a mean 2.4 fold reduction in surface FcμR expression whereas a 3.9 fold increase in surface IgM expression compared to unstimulated cells (P<0.001 and P=0.016, respectively). Since IL-4 is produced by T cells, which typically interact with CLL cells in LNs, we next compared surface FcμR and IgM expression in two paired LN and PB CLL samples. Interestingly, the expression levels of both FcμR and IgM were downregulated in LNs, whereas no difference was detected in the expression of surface IgM. To further understand the mechanisms through which IL-4 regulates BCR signaling, we compared BCR signaling capacity of CLL cells cultured for 48 hours in the presence of IL-4.
Summary/Conclusions: These data show that FcgR is a negative regulator of BCR signaling in CLL cells. Overexpression of FcgR could be at least in part responsible for the reduced BCR signaling capacity of PB CLL cells. FcgR 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 EFFECT ON WITH AGE: MARKERS OF IMMUNOSENESCENCE?

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Background: Aging is characterized by a progressive decline in immune surveillance that favors tumor development in older patients. One mechanism used by malignant cells to escape immune surveillance is the upregulation of immune checkpoint inhibitors. Another process associated with aging is genetic or epigenetic modifications of tumor suppressor genes (TSGs).

Aims: Investigate the role of a correlation on BCR signaling 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 different lymphocytes subsets for their potential impact on 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 purchase of each lymphocyte subpopulation between 95%-99%. PD-1 (PDCD1), PD-L1 (CD274), IL-4, IFNG, BACH2 and PRDM1 mRNA transcripts were quantified using qRT-PCR. BACH2 and BLIMP1 (PRDM1) protein expression were examined by Western blotting.

Results: Blood samples were obtained from 60 healthy volunteers and 41 untreated B-CLL patients (median age: 67yo). Healthy donors (HD) between the ages of 20 to 90 years subdivided into <50 yrs (median: 36yo) and >50 yrs (median: 65yo). BACH2 mRNA expression in the HD groups is significantly down-regulated in CD4+, CD8+ T cells and CD19+B cells from the older HD group (p=0.0012; 0.0045 and 0.0367, respectively). BACH2 expression was further reduced in CD4+, CD8+ T cells and CD19+B cells from CLL patients compared to HD well balanced for age (p=0.001; <0.0001 and 0.0043). PRDM1 mRNA expression was inversely correlated with BACH2 in CD4+, CD8+ T cells and CD19+B cells (r=0.61; 0.71 and 0.85, respectively). Curiously, PRDM1 was – as expected - significantly up-regulated in CD4+ and CD8+ T cells (p=0.0034; p=0.0017) from B-CLL patients but not in their leukemic B cells. Western blotting analysis demonstrated that BACH2 and BLIMP1 (PRDM1) protein expressions in the T and B cell subpopulations were significantly correlated with transcript expression. BACH2/PRDM1 gene expression was up-regulated in CD4+ producing CD4+ T cells. We also observed that BACH2 down-regulation is 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-1 mRNA expression was correlated with increased age in HD B cells (p=0.04) with a further increase detected in CD8+ cells (p=0.001). We also observed an inverse correlation between BACH2 and PD-1 in CD4+, CD8+ T cells (r=0.62 and 0.68), and between BACH2 and PD-L1 in CD19+B cells (r=0.66).

Summary/Conclusions: These data suggest that down-regulation of BACH2/PRDM1 and up-regulation of PD1/PD-L1 mRNA expression in major 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)

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Background: The different biological mechanisms leading the clinical progression of CLL from early stages are currently not fully elucidated. Different prognostic factors that show a higher probability of progression, such as increased number of circulating mature B cells (BC), have been associated with the clinical course of CLL. Nevertheless, the bulk of CLL patients 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 CD137 compared to T cells from healthy individuals. Taking this into account, we hypothesize that changes in the microenvironment, and particularly in T-cell exhaustion component, are contributing to the clinical progression of CLL.

Aims: In order to explore the role of the immune system in the progression of CLL we studied the immunophenotype of T cells from CLL patients using paired samples at diagnosis and progression.

Methods: A total of 14 CLL patients (median age, 69 years; median time of progression of 29.5 months) and 6 patients diagnosed with CLL that did not experience clinical progression during a median follow up of 34 months were included in the study. Multicolor flow cytometry was performed in matched samples at two time-points: diagnosis and progression before treatment or diagnosis and follow-up. We studied T-cell differentiation status based on CD45RA and CCR7 expression and the inhibitory receptors PD1, CD244, CD160, LA93, TIM3 and CTLA4. We also analyzed the expression of the transcription factors BACH2/PRDM1 and Eomes.

Results: We observed a significant increase in CD8+ absolute numbers (P=0.0107) and a significant decrease of the CD4:CD8 ratio (P=0.0012) with progression. T cells increased their effector memory (EM) (CD45RA-CCR7-) phenotype during progression (EM CD4+ P=0.0353; EM CD8+ P=0.0023), PD1 expression was significantly increased during progression in absolute numbers and percentages (PD1+CD8+ cell subset (P=0.0169) as well as in the PD1+ EM subset (EM PD1+CD4*; EM PD1+CD8* P=0.0024). Interestingly, we did not observe these changes in CLL patients that did not progress where the absolute numbers of cells expressing PD1 were either diminished or maintained during the follow-up, pointing out an important role of PD1 in regard to CLL progression. We observed that the percentage of CD3+CD8+ T cells and CD4+CD160 were higher at the time of progression, especially for CD244 (P=0.0078). Moreover, the co-expression of these markers with PD1 was found on CD8+ T cells and its percentage was increased during progression (P=0.0078). Among the differentiation subsets, the EM and EM CD45RA* (TEMRA) CD8+ T cells expressed the highest percentages of CD244 and CD160. We did not observe changes in LA93, TIM3 and CTLA4. T-bet and Eomes are essential to regulate T-cell differentiation and their expression has also been associated with a progenitor (T-bethi PD1hi) or a terminal (Eomeshi PD1hi) exhausted phenotype in chronic viral infections. The percentage of CD8+ T cells expressing the exhausted signature (Eomeshi PD1hi) was significantly increased during progression (P=0.0186 and P=0.0286, respectively) whereas T-bet expression was more stable.

Summary/Conclusions: T cells from patients with progressed CLL show a more severe exhausted phenotype compared to diagnosis, which is characterized by an effector memory subset with higher expression and co-expression of PD1, CD244 and CD160, as well as higher levels of the transcription factor Eomes, indicating that the terminal exhausted phenotype (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 IGDM BUT NOT OF OTHER ASSOCIATED MOLECULES APPEARS TO REFLECT ANTIGEN DROPOUT AND ENHANCE IN CLL PATIENTS ON IBUTINIB THERAPY

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Background: B cell receptor (BCR) signaling through surface IgM (sIgM) is key to the survival and proliferation of normal and chronic lymphocytic leukemia (CLL) cells, and can be targeted effectively by the BTK inhibitor ibrutinib. Chronic exposure of the BCR to (super)antigen leads to downmodulation of sIgM,
but not of sIgD, levels and signaling capacity. This is evident in the circulating CLL B-cells which are characterized by variably reduced sIgM levels/signaling. The variability influences outcome and cases with relatively higher sIgM levels/signaling capacity, but not sIgD, 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 T-cell compartment and function of sIgM 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 sIgM, sIgD, CD19 and other surface markers was assessed by flow cytometry. Signaling capacity following sIgM 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 sIgM expression. ImmunoSEQ® analysis was obtained from all patients (REC: H22/020). Results: At week 1 of ibrutinib therapy, there was a dramatic increase in the expression of sIgM on the circulating CLL cells (mean fold increase 1.6, P=0.001), while expression of sIgD and CD19 remained constant. At this time point, increased sIgM expression associated with full N-glycan maturation of sIgM heavy-chain, indicative of recovery from antigen engagement at tissue sites. Also, the sIgM levels correlated with increased anti-igM mediated SYK phosphorylation (r=0.64, P=0.03), to indicate functionality upstream of BTK. Sequential assessment at month 1 and 3 revealed that sIgM levels were similar to that observed prior to therapy, with preserved upstream signaling ability. In marked contrast, the other BCR complex associated molecules sIgD, 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 sIgM expression and function from other BCR-complex associated molecules. In the initial phase, the increased sIgM expression and maturation, with no changes of other BCR-associated molecules, appears consequent to lack of antigen encounter, likely due to inhibition of chemokine-mediated entry to tissue sites. In the later phases the circulating CLL cells will suffer lack of tissue derived protective stimuli. In their absence, CLL cells will reduce expression of several markers and cell size, possibly explained by autophagic mechanisms aiming to protect the circulating CLL cells from death unless ibrutinib therapy is withheld.

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TRB REPertoire PROFILING OF T-Cell-1 TRANSGENIC MICE USING NOVEL NGS TECHNOLOGIES REVEALS OLIGOClonAL EXPANSIONS: SIMILARITIES WITH CHRONIC LYMPHOcytic LEUKEMIA

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Background: Findings from independent studies reported that the BcR pathway and antigen stimulation occupy a central spot in the development of leukemia in the Eμ-TCL-1 transgenic (tg) mouse, as in the case of chronic lymphocytic leukemia (CLL). Recently, the detailed characterization of the T-cell receptor beta chain (TRB) gene repertoire in patients with CLL revealed gene expression biases and oligoclonality. These characteristics strongly suggested that not only leukemic B cells, but also T cells are selected by antigenic elements that may shape the T-cell compartment in TCL-1 mice, similar to human CLL. This result further support the notion that this mouse model closely resembles CLL, at least from an immunogenetic perspective.

E998

ROLE OF THE COMBINATION MEK1/2 INHIBITOR BINimetinib AND AKT INHIBITOR MK2206 IN CLL

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Background: Clinical trials of ibrutinib and idelisib demonstrated the efficacy of B-cell receptor-targeted therapies for CLL. We sought to investigate the efficacy of targeting both the BcR and the MAPK-ERK1/2 signaling pathways. Aims: To evaluate the role of targeting the Ras-Raf-MEK1/2-ERK1/2 together with the PI3K-AKT pathways as a potential novel approach in treating chronic lymphocytic leukemia. In particular, assessing the efficacy of MEK1/2 inhibitor, binimetinib (MEK162), in combination with either a PI3K inhibitor, 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 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.

Figure 1. Results: MK2206 is effective against CLL cells co-cultured with stromal cells in a dose dependent manner. It was also observed that the primary CLL cells co-cultured with the CD40L-expressing stroma were significantly more sensitive to MK2206 than to idelisib (Figure 1A). No cytotoxic effects of binimetinib...
were observed while the combination with MK2206 was significantly more effective than either alone, suggestive of synergistic action between the two drugs (Figure 1B). The analysis of binimetinib at 20µM with idelalisib failed to demonstrate any additive effects or suggestion of synergy between the two drugs (Figure 1C). Binimetinib treatment led to an increase in the activity of AKT and a decrease in ERK1/2 phosphorylation. MK2206 completely abrogated the activation of AKT and MCL-1 phosphorylation when combined with binimetinib (Figure 2A). Although we observed a reduction in AKT phosphorylation following idelalisib alone, it had no effect on the levels of AKT activity induced by binimetinib or the levels of phosphorylated MCL-1 protein. This result was irrespective of the dose of idelalisib used (Figure 2B). We explored the possibility that protein kinase C (PKC) may be involved in binimetinib-induced AKT phosphorylation. Using the pan-PKC inhibitor GF109203X (GFX), we demonstrated that inhibition of PKC significantly reduces binimetinib-induced phosphorylation of AKT with no effect on the activity of ERK1/2-MAPK (Figure 2C). These data suggest a role for PKC in the regulation of AKT activity in CLL cells.

Summary/Conclusions: The combination of binimetinib and MK2206 in vitro has been shown to be effective strategy to treat primary CLL cells. The western blot data reinforce that the increased activity observed in AKT activity in CLL cells following binimetinib treatment is independent of the idelalisib and totally abrogated by MK2206. This PI3-kinase independent regulation may be regulated by other factors as well, most probably by PKC.

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-ar-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: 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. Similiar effects were observed by targeting the PI3K/Akt pathway with Idelalisib. We then investigated whether targeting RhoA- and Ras-dependent signaling could modulate HIF-1α also at the SC level. Simvastatin and BAY87-2243 effectively inhibited HIF-1α expression both in SC lines and in patient-derived SC. Moreover, simvastatin significantly reduced the secretion of CXCL12, which is a known transcriptional target of HIF-1α.

Summary: Our data demonstrate that the targeting of HIF-1α and its regulatory pathways, both at the tumor cell and at the SC level is an appealing strategy to overcome the microenvironment-mediated protection to spontaneous and fludarabine-induced apoptosis in CLL cells.

E1000
THE ROLE OF GENETIC-BASED PROGNOSTIC FACTORS IN PREDICTING MINIMAL RESIDUAL DISEASE NEGATIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS TREATED WITH FLUDARABINE, CYCLOPHOSHAMIDE AND OFATUMUMAB

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et al., 2013), the high and intermediate risk groups (del17p/TP53/del11q+ or del11q/NOTH1/SF3B1) showed a significantly lower probability of achieving a MRD negativity (36%, 10/28) than the low and very low risk groups (<10% 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%) MDR- 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/TP53mut/del11q, the association with a deep MRD negativity got stronger (12 MRD-22 MRD+ 36%, 47/135 cases), with a median time of 77.5 months [22-291] from the diagnosis. Karyotype was completed in 195 CLL patients harboring a 17p deletion, i.e. 17p- CLl. Gains and losses of chromosome 17 were identified by FISH (40) and/or flow cytometry. A multivariate model including FISH lesions, gene and IGHV mutations supports the independent role of FISH and IGHV profile in predicting MRD negativity by flow and ROC-PCR, respectively.

Summary/Conclusions: In CLL patients treated with the FCO combination (LLC0911 Gimema trial), a greater MRD negativity by flow cytometry (82%) can be predicted by the FISH profile: 74% in patients without del17p/del11q 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.

### E1002

**THE MICROENVIRONMENT REGULATES THE EXPRESSION OF MIr-21 AND TUMOR SUPPRESSOR GENES PTEN, PIA3 and PDCD4 THROUGH THE MICROENVIRONMENT REGULATES THE EXPRESSION**

**Aims:** First, we observed that miR-21 expression was significantly higher in miR-21 positive cases (74%) compared to miR-21 negative cases (26%) (p=0.0375), the presence of unbalanced translocations in 17p- CLl (17q) confers a shorter OS than the other 17p abnormalities. In addition, the gain8q remained significant for OS.

**Summary/Conclusions:** Among the high risk group of 17p- CLl, (17q) confers a shorter OS than the other 17p abnormalities. In addition, the gain8q aggravates the outcome as well as the presence of additional unbalanced translocations. These results confirm that patients with 17p- CLl have a variable clinical course and highlight the relevance of conventional karyotyping to identify the alterations that modulate the prognosis within these patients.

**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 microenvironment. 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 pathogenesis 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 stimulation, 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 metastasis; its overexpression in CLL has been associated to refractoriness to flu-}

**E1001**

**ISOCHROMOSOME 17Q, UNBALANCED TRANSLocations AND 8q GAIN REPRESENT ADVERSE PROGNOSTIC FACTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH 17p DELETION. A GFCH STUDY**

**Aims:** To identify the clinical parameters associated with the time to first treatment (TTFT) of patients with 17p deletion. Despite the high frequency of 17p deletion (12-17% of all CLL patients), it is still unclear whether del17p is an adverse prognostic factor. We collected data from a multicentric and retrospective cohort of 195 CLL patients harboring a 17p deletion. Gains and losses of chromosome 17 were identified by FISH, with an informative conventional karyotype and the additional aberrations could influence the prognosis. Patients with 17p- typically progress quickly and are refractory to most conventional therapies.

**Summary/Conclusions:** The presence of additional unbalanced translocations in 17p- CLl (17q) confers a shorter OS than the other 17p abnormalities. In addition, the gain8q aggravates the outcome as well as the presence of additional unbalanced translocations. These results confirm that patients with 17p- CLl have a variable clinical course and highlight the relevance of conventional karyotyping to identify the alterations that modulate the prognosis within these patients.

**Background:** Chromosomal abnormalities are present in about 80% of CLL. Among them, the loss of the short arm of the chromosome 17 (17p-), unassociated with del17p, is frequently accompanied by genomic complexity. Patients with 17p- typically progress quickly and are refractory to most conventional therapies.

**Summary/Conclusions:** The presence of additional unbalanced translocations in 17p- CLl (17q) confers a shorter OS than the other 17p abnormalities. In addition, the gain8q aggravates the outcome as well as the presence of additional unbalanced translocations. These results confirm that patients with 17p- CLl have a variable clinical course and highlight the relevance of conventional karyotyping to identify the alterations that modulate the prognosis within these patients.

**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 anti-IgM (LY294002), 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 microenvironment found in proliferation centers. After 48 hours CLL cells were harvested to analyze cell viability, cell proliferation and mRNA expression. Expression levels of primary miR-21, miR-21, PTEN, PDCD4 and PIA3 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 expression were significantly increased upon BCR stimulation, which was enhanced by ectopic expression of ZAP-70. We also observed that inhibition of both MAPK and STAT3 pathways impairs the regulation of miR-21 expression after ZAP-70 activation. Moreover, the induction of miR-21 expression after ZAP-70 activation also induced downregulation of the tumor suppressor genes PTEN, PDCD4 and PIA3. In conclusion, miR-21 may play a role in the crosstalk between CLl cells and the microenvironment.

**Summary/Conclusions:** Inclusion, stimuli from the microenvironment are capable of regulating expression of miR-21 and tumor suppressor genes
IMPACT OF RECURRENT MUTATIONS ON PROGRESSION-FREE SURVIVAL IN CLL PATIENTS TREATED WITH FRONT LINE RITUXIMAB-BASED REGIMENS

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Background: Regimens consisting of rituximab and DNA-damaging drugs represent an important therapeutic option for patients with chronic lymphocytic leukemia (CLL). Up-to-date studies including clinical trials agreed upon the adverse outcome of TP53-defective patients that should be provided alternative treatment approaches. Additionally, mutations in NOTCH1 gene were connected with a lack of benefit from rituximab added to chemotherapy. A potential impact of other mutations commonly occurring in CLL patients remains less clear, namely regarding a role in relapse development.

Aims: (a) to assess impact of mutations in ATM, SF3B1, NOTCH1 and BIRC3 genes on progression-free survival (PFS) in CLL patients treated with front line rituximab-based regimens, and (b) to analyze clonal evolution of mutations in relapse.

Methods: We analyzed 53 CLL patients administered first line regimens (R, FCR (fludarabine, cyclophosphamide, rituximab) or Q-FCR (FCR with reduced doses) or BR (bendamustine, rituximab); all harbored intact TP53 gene as assessed by FISH and the yeast functional assay; 46/53 (87%) had unmutated IGHV. The next generation sequencing using MiSeq (Illumina) was done in 48 patients (100%) and 41 relapsed samples using three and five gene panels: ATM (exons 2-6; median coverage (MC) 8100), 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 analyses (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-therapy analysis, we identified 23 patients with one disrupted gene and 7 patients with two disrupted genes; the rest of the cohort (n=6) did not have any mutations (wt). ATM was the most frequently affected gene (n=15; only mutations with predicted functional impact considered), followed by SF3B1 (10 cases; hot-spot mutations), NOTCH1 (7 cases; all deletion c.7541T>7542) and BIRC3 (5 cases; frame-shift mutations). We did not observe significant differences in PFS among the employed regimens; the median PFS of FCR (used in 30 patients; median: 4 cycles), 15 m for Q-FCR (15 patients; 4 cycles); and 14 m for BR (8 patients; 3.5 cycles); P=non-significant (ns). In univariate analyses of individual affected genes, we did not observe an adverse impact of mutations on PFS: ATM-mut median 19 m vs 14 m in wt patients; SF3B1-mut 19.5 m vs 16 m; NOTCH1-mut 32 m vs 15.5 m; BIRC3-mut 16 m vs 16 m (all analyses P=ns). In a more detailed analysis, we observed a higher percentage of patients with double ATM mutations (in seven patients) and one case of ATM mutation from two (from 2.3% to 7.7%, and 5.4% to 6.54%) and ATM mutations in two cases (8% to 15% and wt to 18%), as well as more complex changes involving both increase and decrease of mutations in BIRC3 gene in three patients.

Summary/Conclusions: Our pilot analysis with limited number of samples does not indicate an adverse impact of studied mutations in rituximab-based regimens. Some relapsed samples showed quite distinct mutation profile. Supported by projects AZV 16-32743A, MUNI/A/1106/2016 and FNBr, 65269705.

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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 CD5. Here, we present a study that correlates the interaction of CXCR4 with CD5 expression in CLL cells from patients treated with rituximab, as well as their meeting with the leukemic cells in vivo. We observed that CXCR4-defective cells tend to have a higher CD20 expression than CLL cells circulating in the peripheral blood without contact with immune niches (CXCR4high/CD5dim; Pavlova et al., 2016). We hypothesised that the higher levels of CD20 on CXCR4dim/CD5dim cells make them the primary target for RTX in vivo, since the cell line is rendered CD20 expresser by the RTX (Kroeger et al., 2001). We analysed blood samples obtained from CLL patients treated with RTX as a single agent and indeed, we observed that RTX preferentially and nearly completely eliminates the CXCR4dim/CD5dim subpopulation after the first RTX dose (8.3% pre-RTX vs 2.1% post-RTX, P<0.0001). We further showed that RTX down-regulates signaling efficiency of the CXCR4 receptors in CD20+ subpopulation, since CD20 was proposed to play a role in BCR signalling. We observed that CXCR4dim/CD5dim 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, CXCR4dim/CD5dim cells also have higher levels of CD19 (1.8-fold, P<0.0001), which is an important component of BCR complex that augments signal transduction. Furthermore, we demonstrated that CXCR4dim/CD5dim cells have higher phosphorylation of several proteins involved in PI3K/BCR/NFkB signalling pathway (P<0.05) compared to CXCR4high/CD5dim cells obtained from the same patient. This led us to hypothesize that the down-regulation of CXCR4 expression 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 CXCR4dim/CD5dim CLL subpopulation 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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REGULATION OF BCR SIGNALLING IN CHRONIC LYMPHOCYTOCYC LEUKAEMIA: ROLE OF E3 UBIQUITIN LIGASE C-CBL

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Background: In normal B cells, the E3 ubiquitin ligase Cbl (c-CBL) is involved in the ubiquitin-dependent Lyn degradation and in the down-regulation of BCR signalling. 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 ligase activity thus prohibiting lysosomal or ubiquitin/proteasome-mediated degradation of tyrosine kinases and thereby unleashing tyrosine kinase signaling. 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 multiprotein complex, associated with several proteins, (CXCR4, BIRC3, HS1 and SHP-1L). In particular, Hsp90 appears tightly bound to Lyn c-Cbl complex structure, facilitating Lyn translocation to the cytosol, 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 are associated with BCR-mediated cell survival 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 is involved in the ubiquitin-dependent Lyn degradation and in the down-regulation of BCR signalling. Moreover, the studied 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 dysregulation of neoplastic clone. In CLL cells c-Cbl is overexpressed with respect to normal B cells, and upon BCR engagement it undergoes Y700 phosphorylation. However, c-Cbl is unable to stably interact with Lyn suggesting an altered c-Cbl function that contribute to affect cell homeostasis.

E1006

ACTIVATION OF SHP-1/PP2A PATHWAYS TRIGGERS APOPTOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Background: CLL B cells inability to reach programmed cell death is due to intrinsic defect and extrinsic factors. Among the intrinsic fault there is the misregulation of the phosphorylation pattern. Reversible protein phosphorylation is a fundamental post-translational modification by which virtually all cellular events are regulated. The crucial players involved in this dynamic process are protein kinases and protein phosphatases, which are placed at the different levels of cellular signaling. The Src Family Kinase (SFK) Lyn is a key factor in the dysregulation of survival and apoptotic pathways of malignant B cells in CLL. One of the effects of Lyn’s action is the spatial and functional segregation of the tyrosine phosphatase SHP-1 into two pools, one beneath the plasma membrane in an active state promoting pro-survival signals, the other in the cytosol in an inhibited conformation and unable to counter the elevated level of cytosolic tyrosine phosphorylation.

Aim: Because CLL is characterized by a high level of Lyn-dependent tyrosine phosphorylation in the cytosol, we focused our attention on compounds capable of directly or indirectly driving the activation of SHP-1 which in turn could counter the action of Lyn and induce cell demise. The goal is to discover new therapeutic strategies to defeat a still incurable disease as CLL.

Methods: B cells were isolated from 37 CLL patients. Freshly isolated CLL cells incubated with increasing concentrations of nintedanib (0-24 μM) and MP07-66 (2,2-diethoxyethyl[di-(hexyloxy)phenyl(methyl)]amine) for 24 and 48 hours with/without a ligand of Mesenchymal Stromal Cells (MSCs). Caspase-dependence was demonstrated using the pan-caspase inhibitor z-VADfmk. CLL B cells viability was tested by Flow Cytometer with Annexin V/PI test and PPP2Ac expression and phosphorylation were evaluated by Western Blotting.

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 phosphorylated (at Ser591), and inhibited, form of SHP-1 and to induce apoptosis, depending on the caspase activation, after 24h and 48h at marked level. Interestingly, we recently demonstrated that Ser591 phosphorylation of SHP-1 could be dephosphorylated by PP2A. In this scenario, the restoration of PP2A activity by a finkglobin, a drug devoid of immunosuppressive action, called MP07-66, and the subsequent dephosphorylation of PP2A substrates, was shown to trigger apoptosis, like nintedanib, in a caspase-dependent manner. Since our data suggest that the activation of either PP2A or SHP-1 triggered by specific small molecules caused stimulation of each of events 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 MASC layer, showing the capability of these treatments to counteract the protective action of tumor microenvironment.

Summary/Conclusions: In conclusion, our findings indicate that phosphatase activators may represent a new weapon against this form of leukemia. Overall, these data corroborate the hypothesis that the inhibition of PP2A is central to CLL cell viability and that its activation is facilitated by the supportive action of SHP-1, as demonstrated by the effect produced by the simultaneous use of the respective activators.

E1007

TARGETING NANOPARTICLES TO CHRONIC LYMPHOCYTIC LEUKAEMIA: EXPLOITING THE PROPERTIES OF CXCR4

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Background: Nanoparticle carriers of therapeutic agents (‘drug delivery vehicles’) can be used to deliver drugs to specific cells through the incorporation of a ‘targeting ligand’. Targeting provides the therapeutic benefit of achieving high local drug concentrations while reducing off-target effects against other cells; the combined ligand/delivery vehicle system can also be manipulated to determine the uptake pathway or modulate biological effects. The CXCR4 chemokine-receptor is an attractive target for drug delivery vehicles. It is overexpressed in cancers including chronic lymphocytic leukaemia (CLL) (Doman ska et al., 2013) and binding to its ligand (CXCL12) may induce proliferation, survival or entry into protective cellular niches (Ganju et al., 1998). Targeted nanoparticles that can bind and antagonise CXCR4 could therefore allow specific drug delivery to cancer cells while simultaneously blocking CXCL12-induced chemoprotection.

Aims: A drug-design strategy was developed to synthesise and evaluate a novel CXCR4 targeting motif (BAT1) with structural similarity to Plerixafor, a CXCR4-antagonist in clinical use. A key design principle was to incorporate a polyethylene glycol (PEG) tether with a functional end-group to provide an attachment point for cargoes, particularly liposomes. The evaluation aim was to assess the effectiveness of BAT1 to deliver a chemotherapy cargo to CLL cells within an ex vivo culture system.

Methods: A three-step synthesis was used to generate BAT1 (Figure 1A); its structure and purity was confirmed using NMR, MS and HPLC. Bioactivity testing employed primary CLL lymphocytes. Assays performed: CXCR4 binding-affinity (flow cytometric competition assays), cell-binding characteristics (immunocytotlourescence) and blockade of CXCL12-induced signalling (immunoblot). Initial targeting assay used a fluorescent label (Cy5) conjugated to the functional PEG tether. Cholesteryl chloroformate was then selected to conjugate BAT1 to PEGylated liposomes.

Results: The binding affinity of BAT1 (Figure 1B) was demonstrated using competition assays (CXCL12, anti-CXCR4 ab, and the bis(cyclam) drug Plerixafor). The studies confirmed BAT1 had high affinity for CXCR4 receptors expressed on primary CLL cells. Immunocytolourescence comparison with its native ligand confirmed binding of BAT1 to the CLL cell surface, while immunoblotting demonstrated blocking of CXCL12-induced signalling (Figure 1C and 1D). The fluorescent moiety Cy5 was covalently linked to the PEG moiety as a test-cargo, demonstrating that binding affinity was retained in the presence of a cargo and that the drug competed for CXCR4 binding with related bis(cyclam) drugs. This work has been extended to attach BAT1 to liposomes, with present work optimising liposome characteristics for binding and uptake by CLL and the delivery of cytotoxic payload.

Summary/Conclusions: A novel bis(cyclam) CXCR4 antagonist and targeting motif (BAT1) has been synthesised. BAT1 demonstrates high affinity for the CXCR4 receptor, supporting targeted delivery to CLL cells. Receptor binding is associated with simultaneous blockade of CXCL12-mediated signal initiation and effect, and therefore biological modulation of target cell behaviour. BAT1

Figure 1.

Summary/Conclusions: A novel bis(cyclam) CXCR4 antagonist and targeting motif (BAT1) has been synthesised. BAT1 demonstrates high affinity for the CXCR4 receptor, supporting targeted delivery to CLL cells. Receptor binding is associated with simultaneous blockade of CXCL12-mediated signal initiation and effect, and therefore biological modulation of target cell behaviour. BAT1
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. Using qRT-PCR we detected ROR1 mRNA expression in five patients tested consecutively in several time-points. We distinguished malignant population from healthy B-cells. Using qRT-PCR we 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 ibritinib or ibrutinib and idelalisib we observed steep increase of ROR1 expression compared to patients treated with a single agent regimen.

Summary/Conclusions: R101 protein remains detectable on CLL cells during disease course even in complete remission. R101 mRNA levels are highly influenced by therapy administration especially in the case of treatment with BcR inhibitors.


E1010

NORMAL SERUM PROTEIN ELECTROPHORESIS IDENTIFIES AN ANGELI STAGE II-RECLUSION PROGNOSIS GROUP AMONG IGHV MUTATED PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA, WITH A MEDIAN TFS OVER 18 YEARS

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Background: Approximately 36% of patients with chronic lymphocytic leukemia (CLL) have normal serum protein electrophoresis (SPE) either with hypermagnolubinenia or with monoclonal immunoglobulin (Ig) peak. In this study, we compared locally recruited patients with normal and abnormal SPE.

Aims: The aim was to identify prognosis parameters.

Methods: A total of 189 patients (132 abnormal SPE and 57 normal SPE) were included. The patients' diagnoses were performed between 1980 and 2015. Prognosis parameters investigated were IGHV mutation status, presence of SF3B1, NOTCH1 or BIRC3 mutations (determined by high throughput and Sanger sequencing), and cytogenetic abnormalities such as del17p, del11q, del13q and trisomy 12 (assessed by standard cytotype, FISH analysis and QMPSF).

Results: In this series, 73%, 19% and 8% of patients were at Binet stage A, B and C respectively, and 30% had a normal SPE at diagnosis. Ninety six percent of patients with normal SPE were at Binet stage A, versus 63% of patients with abnormal SPE (Chi2 test : p<10^-4). Median lymphocytosis at diagnosis was lower in patients with normal SPE (12.82 G/L versus 19.54 G/L in abnormal SPE, respectively; p=0.010). In patients with normal SPE, we found that 58% of cases with normal SPE had a good prognosis profile (mutated IGHV and/or isolated del13q, with no other genetic abnormality detected), meanwhile 65.2% of patients with abnormal SPE exhibited at least one poor prognosis marker (unmutated IGHV, mutation of SF3B1, NOTCH1, del17p, del11q or trisomy 12, Chi2 test : p<10^-4). In patients with normal SPE, only 3.5% cases were SF3B1 mutated against 15.2% in case of abnormal SPE (Chi2 test : p=0.002). Among other strong differences, 10.5% patients with normal SPE had a trisomy 12 against 18.2% for abnormal SPE. Isolated del13q was found in 38.6% and 21.2% of cases with normal and abnormal SPE respectively. Mutated IGHV status was found in 65% in normal SPE and 56.5% with abnormal SPE. Compared to the whole series, IGHV repertoire analysis shows bias in IGHV1-2, and IGHV4-3 rearrangements, with decreases usage of IGHV3-21 and IGHV3-48. Treatment free survival was markedly increased in patients with normal SPE (median of 10.0 years versus 3.0 years for normal and normal SPE respectively, Kaplan-Meier, log rank test: p<0.01). In patients with normal SPE, death or progression (D or P) occurred in 14.5% of patients vs 53.8% of patients with abnormal SPE (log rank test: p<0.001).

Results: In this study, we aimed to differentiate ROR1 expression 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 ibritinib and idelalisib and 4 patients treated with ibrutinib or idelalisib. ROR1 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-flow colour flow cytometry (modified MRD protocol: CD45/CD3/CD19/CD5/CD81/CD79b/CD22/ROR1) in all samples. To quantify ROR1 mRNA expression changes within individual patients we performed qRT-PCR in separated CLL cells (>95% CD19+CD5+). CLL cells from patients in remission were separated immunomagnetically (Whole Blood Anti-ROR1 MicroBead Kit, Miltenyi Biotec).

Results: Using multicolour flow cytometry we confirmed ROR1 antigen/protein expression on CLL cells. We focused on RNA samples. The 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 ibrutinib and idelalisib we observed steep increase of ROR1 expression compared to patients treated with a single agent regimen. 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 clear cells (PBMCs) of untreated CLL patients (Rai stages 0-IV) and healthy donors. Using qRT-PCR we detected ROR1 mRNA expression in five patients tested consecutively in several time-points. We distinguished malignant population from healthy B-cells. Using qRT-PCR we detected ROR1 antigen/protein expression 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 antigen 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 ibrutinib and idelalisib we observed steep increase of ROR1 expression compared to patients treated with a single agent regimen.

Summary/Conclusions: R101 protein remains detectable on CLL cells during disease course even in complete remission. R101 mRNA levels are highly influenced by therapy administration especially in the case of treatment with BcR inhibitors.
median TFS of 4 years (log rank test: \( p = 0.0003 \)). Thus, patients with normal SPE and IGHV mutated status constitute a group with excellent prognosis.

**Summary/Conclusions:** In conclusion, normal SPE was associated with good outcome with decreased accumulation of side genetic events (in particular, SF3B1 mutations). This analysis shows a bias in IGHV repertoire according to SPE status. These results also clearly suggest that patients with a normal SPE and mutated IGHV have an extremely quiet CLL natural history. This could be either due to the weaker activity of the disease and/or to the absence of adverse consequences of a concomitant paraprotein.

**E1011**

**HSP70 EXPRESSION IS MODULATED BY ITS MASTER REGULATOR HSF1 VIA PHOSPHOAKT AND PI3K/AKT/MTOR PATHWAYS IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background:** The search for molecules involved in apoptosis resistance/ increased survival of B cells from Chronic Lymphocytic Leukemia (CLL) is still ongoing since this disease remains not definitively understood. We recently found that the Heat Shock Protein of 70kDa (HSP70), expressed in response to a wide variety of stress signals and allowing cells to survive to lethal conditions, was particularly overexpressed in neoplastic B cells from CLL. Moreover, the Heat Shock Factor 1 (HSF1), the major responsible for the transcription of HSP70, is itself overexpressed in CLL and strictly correlated to HSP70. In response to stress, HSF1 becomes phosphorylated, forms homotrimers, binds DNA and activates heat shock gene transcription. HSF1 is regulated by a fine balance of activatory/inhibitory phosphorylations mediated by kinases belonging to pathways triggered by RAS (i.e. PI3K/AKT/mTOR and RAF/MEK/ERK). Aims: Since HSP70 is overexpressed in CLL neoplastic B cells and most of “HSF1-1phosphorylating agents” 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 evaluated the activation/expression of key signalling proteins. Herein, we focused on HSP70, AKT-Ser473, mTOR-Ser244, GSK3α/b-Ser21/9, CDK2, CREB-Ser133, and p38MAPK-Thr180/Thr182, SAPK-JNK-Thr183/Tyr185 and PDK1-Ser241. Cluster and separation analyses have been performed.

**Results:** We divided our patients in HSP70-high and HSP70-low considering as cut-off the value of the median of HSP70 expression levels calculated by RPPA and demonstrated that the examined proteins behave in a different way between patients expressing high or low levels of HSP70. HSP70-high patients present high AKT-Ser473, an inhibitor of GSK3α/b, that in the inhibited form, prevents HSF1 inhibition. By contrast, HSP70-low patients have high MEK1/2-Ser217/221 and ERK-Thr202/Tyr204, known to negatively regulate HSF1. Intriguingly, p38MAPK-Thr180/Tyr182 which has been described to both activate and inhibit HSF1 at different sites, is overexpressed in those patients presenting low levels of HSP70.

**Summary/Conclusions:** These data would suggest that, in CLL, HSP70 expression is regulated by the modulation of HSF1 activity through the activation of one or the other way triggered by RAS. In particular, an activation of the PI3K/AKT/mTOR pathway leads, as result, to a higher expression of HSP70 while an activation of the RAF/MEK/ERK signalling rather results in HSP70 down regulation. The dissection of signalling pathways connected to HSP70-HSF1 axis in CLL will contribute to define the biology and understand the pathogenesis of this disease.

**E1012**

**THE INTERPLAY BETWEEN TH17 AND TREGS: A NEW IMMUNOSUPPRESSIVE INSIGHT IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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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 (10ng), followed by a 5 h stimulation with PMA, Ionomyc and Monensin (PIM), or with anti-CD3/CD28 PE or anti-CD4/CD28 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:** 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 deprivation of IL-17A-secreting cells. The frequency of IFN-γ-producing T cells resulted statistically significant increased in patients with 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\(^\text{hi}\)FoxP3\(^+\) cells (Tregs) in CLL samples, with a statistically significant increase in Tbet+ Tregs, RORct+ Tregs and GATA3+ Tregs subpopulations (Figure 1).

**Figure 1.**

**Summary/Conclusions:** Our results reported a down-regulation of IFN-γ and IL-4 producing T cells, associated to an increased frequency of Tregs and their subsets in CLL patients, probably trying to overcome the deficit of effector T cells. On the other hand, we observed a rise in IL-17A secreting T cells related to the increased IL-23 production by dendritic cells in order to restore TH17 pool, without changing the percentage of IL-17A+IL-10+ cells. These data support the idea of the protective function of TH17 that show an effector and not a regulatory T phenotype. Starting from these observations, this study could pave the way to further researches and applications in the comprehension of the biological and regulatory mechanisms of TH17 and Tregs, supporting the study of a pioneering anticancer therapy in CLL.

**E1013**

**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 mutational status detected by deep sequencing in a cohort of 138 untreated 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 TrueSeq Custom Amplicon

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containing 13 genes and covering 28,099 bases. Pair-end sequencing was performed with MISEQ v2.2 chemistry, and a mean depth of 96× across the base was obtained. Every patient underwent, at baseline, a flow cytometry characterization with a panel including (si)gκ, (si)gλ, CD19, CD5, CD11b, CD81, CD10, CD79b, CD29, CD38, FMC7, CD22, CD45, CD103, CD11c, CD25, ZAP70, CD11a, and CD24. CD4K 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 preponderance, the median follow up time of our cohort was 43 months (24-104). We found that 38/138 (28%) patients harbored at least one mutation, with NOTCH1 (n=16, 12%), ATM (n=12, 9%), TP53 (n=9, 7%), and SF3B1 (n=8, 6%) being the most commonly mutated genes. Those patients with NOTCH1 mutation showed a lower CD25 expression (24 mean fluorescence intensity units (MFU)) than those without a mutation (43 MFU), p=0.03. We could not validate the recently reported association between the presence of NOTCH1 mutations and a low expression of CD25. In our cohort, the MFI expression in NOTCH1 mutated and non-mutated patients was 163 and 146 units, respectively (p>0.05). We measured CDK4 and CDK6 expression in the CD19+ sorted fraction RNA of 7 NOTCH1 mutated cases and 11 non mutated cases, without finding significant differences (0.26 vs 0.27 for CDK6, 0.025 vs 0.022 for CDK4; p>0.5 in both cases).

Summary/Conclusions: We found a significant inferior expression of CD25 when activating NOTCH1 mutations are present in CLL patients. The relationship found between these two variables, with an inverted direction to that found in physiological conditions, has also been shown in the setting of NOTCH1-mutated T acute lymphoblastic leukemia. In CLL cases, it seems to be independent of CDK4/6 expression, prompting further studies checking CDK4 and CDK6 regulators.

E1014 GENE MUTATIONS ANALYZED BY NEXT-GENERATION SEQUENCING ALLOW US TO DEFINE THE PROGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS WITH EARLY-STAGE DISEASE AND 13Q-DELETION M. Martín1, A. M. Casado2, C. Agüera3, M. Olalla4, M. Barbero5, J. Hernández-Rivas1, A. J. Martín6, R. Benito1, M. J. Vidal-Manceñido2, J. Galende3, J. M. Alonso4, J. M. Hernández-Rivas1

Background: Next-Generation sequencing (NGS) studies have revealed a number of recurrently mutated genes in chronic lymphocytic leukemia (CLL). It is reasonable to argue that evaluation of the newly gene mutations as prognostic markers would help to improve prognostication of CLL patients. Interestingly, gene mutations could help us to refine the prognosis in the group of CLL patients with other prognostic markers associated with good prognosis.

Aims: To analyze the presence of mutations of a panel of genes by NGS and its prognostic impact in patients with CLL, focusing in the patients with good prognosis characteristics.

Methods: Amplicon-based NGS was performed using 454 platform in 147 CLL patients to evaluate the mutational status of genes (TP53, NOTCH1, SF3B1, XPO1, FBXW7 and MYD88). Samples were obtained at diagnosis or before treatment in all cases. 70.1% were Binet A and 53% had 13q deletion (13q-). A cut-off 2% was applied to define variants. All the mutations were validated.

Results: 1. NGS analysis showed that 37.4% of CLL patients (55/147) showed mutations in any of the analyzed genes. The frequency of mutations was 16.3% for NOTCH1, 10.2% for SF3B1, 6.8% for TP53, 4.8% for XPO1, 3.4% for FBXW7, and 1.4% for MYD88. The presence of mutations in any of these genes except MYD88 (mutated CLL) was significantly associated with clinical progression (60.0% for mutated CLL vs. 38.2% for unmuted CLL; P=0.05). Interestingly, mutated CLL patients showed a shorter time to first treatment (TFT) than mutated unmutated CLL patients (30 months vs. 88 months; P=0.006). By contrast, MYD88 mutations were detected in CLL with mutated IGHV and 13q- 2. 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 >40% of cells had a shorter 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). In the group of CLL patients with 13q-, 32.8% of them showed mutations in any of the analyzed genes. Interestingly, CLL patients Binet A with mutations (except to MYD88) showed a shorter TFT than CLL patients without mutations (31 vs 131 months, P=0.001). Besides this, CLL with 13q- as the sole cytogenetic alteration and gene mutations had also a shorter TFT that unmutated 13q- CLL patients (p<0.001).

Summary/Conclusions: 1) CLL patients with mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 show a worse prognosis than CLL patients without mutations. 2) Gene mutations in TP53, NOTCH1, SF3B1, XPO1 and FBXW7 in a low percentage of the cells are associated with a shorter TFT. 3) Among CLL patients with good prognostic characteristics (Binet A and 13q-), gene mutations help us to define the prognosis of the patients.

E1015 ALTERED COMPLEX C5 IS ASSOCIATED WITH COMPROMISED COMPLEMENT ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA A. Braester1,2, M. Barhoum3, R. Michiels1

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Background: The therapeutic monoclonal antibodies used for the treatment of Chronic lymphocytic leukemia (CLL) mediate anti-tumor effects through several independent cell-mediated cytotoxicity (ADCC), and phagocytosis. CDC efficacy thus depends on the expression level of the target B-cell antigen, the integrity of apoptotic cascades within tumor cells, the functional capacity of effector cells, and the availability and activity of the complement (C) system. Published data indicate deficiency of one C protein or more in most CLL patients, as well as additional factors that may affect C activity. The role of structural abnormalities of C complexes in affecting C function has not been investigated.

Aims: To study the structural integrity of circulating C complexes, focusing mainly on C5, and to establish its importance for C activity in CLL.

Methods: Blood samples were obtained from 35 (Binet A and 13q-) CLL patients and 10 healthy controls (HC). Biochemical and haematological parameters, and CLL staging were recorded. The isoforms of two key 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 C3-b and CD59, anti-apoptotic protein dependent cell-mediated cytotoxicity (ADCC), and phagocytosis.

Results: 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 disturbs the C activity need further clarification. Yet, the appearance of abnormal C5 pattern compared to sera from the other subjects' groups. 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 disturbs the C activity need further clarification. Yet, the appearance of abnormal C5 pattern 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 disturbs the C activity need further clarification. Yet, the appearance of abnormal C5 pattern compared to sera from the other subjects' groups.
Chronic lymphocytic leukemia and related disorders - Clinical

E1016
ASSOCIATION OF CGP-STIMULATED KARYOTYPE WITH TIME-TO-FIRST TREATMENT FOR CLL

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.

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<td>WBC “white blood cell; ALC “Absolute lymphocyte count; HGB “Hemoglobin; FISH “fluorescence in situ hybridization.</td>
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Aims: The aim of this study is to report the incidence and the impact of CpG-stimulated karyotype in the treatment of naïve 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-868 (20μg/mL), phorbol 12-myristate 13-acetate (PMA; 0.04μg/ml) and Pokeweed mitogen (PWM; 0.1μg/mL). Banding and analyses were by standard laboratory procedures. Twenty metaphases were analyzed per culture and patients were categorized as having diploid karyotype, a single, 2 or 3 or more(complex) clonal chromosome abnormalities present in more than 1 metaphase by CpG-stimulated karyotype. The frequency and distribution of chromosome abnormalities with other prognostic factors and time-to-first treatment from diagnosis (TTFT) were analyzed (Table + Figure).

Results: The majority (69%) of patients had diploid cytogenetics. Higher-risk prognostic features such as del17p, del11q, unmutatedIGHV and ZAP70 expression were associated with presence of complex karyotype abnormalities. Shorter TTFT from diagnosis was associated with 1, 2, and complex clonal chromosome abnormalities compared to diploid karyotype (<0.005). 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%).

Figure 1. Aims: The aim of this study is to report the incidence and the impact of CpG-stimulated karyotype in the treatment of naïve 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-868 (20μg/mL), phorbol 12-myristate 13-acetate (PMA; 0.04μg/ml) and Pokeweed mitogen (PWM; 0.1μg/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).

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(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 Barcelona-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 Barcelona-Brno prognostic model in predicting TTFT (CLL-IPI, AIC=6060.503 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 Barcelona-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 Barcelona-Brno prognostic model.

Figure 1.

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 Barcelona-Brno biomarkers-only prognostic model.

E1018

PRELIMINARY RESULTS OF S55746/BCL201 (A NEW BCL2 INHIBITOR) IN RELAPSED OR REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS AND EFFECT OF CALIBRATED MODERATE MEAL ON THE PHARMACOKINETICS

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Background: BCL-2 is an anti-apoptotic protein overexpressed in chronic lymphocytic leukemia (CLL). BCL-2 is responsible for apoptosis machinery dysregulation and contributes to chemotherapy resistance. S55746/BCL201 is a novel, oral, selective BCL-2 inhibitor.

Aims: The current first-in-human study evaluates the safety and aims to establish the recommended phase 2 dose; main secondary objectives include evaluation of pharmacokinetics (PK), food effect, pharmacodynamics and preliminary activity in patients (pts) with relapsed or refractory CLL.

Methods: S55746/BCL201 as single agent is being investigated in a phase I (EUDRACT, NCT02920697), open-label, multinational, international dose escalation trial. S55746/BCL201 was initially administered in fasting condition, once daily (in 21-day cycle) until progressive disease (PD) or unacceptable toxicity. A tumor lysis syndrome (TLS) prevention protocol was implemented. After giving informed consent pts could receive 50 to 2000mg according to a modified version of the Continual Reassessment Method for dose allocation process. In the food effect part of the study, 7 non-Hodgkin’s lymphoma patients received a film coated 200mg tablet under fasting condition (i.e. after 10-hour fasting period) and after a calibrated moderate meal the day after. Results: As of February 2017, 12 CLL pts have been treated (median age 67 years [range 52-82]). On these 12 pts, 5 presented a bulky disease, 1 a 17p13 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. A maximum frequent (2x 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-comparitional pharmacokinetic analysis of the food effect cohort (5 assessed pts) demonstrated that S55746/BCL201 PK is not affected by the ingestion of a moderate meal (400-500 kcal with fat contributing to 150 kcal). The median Tmax was delayed from 1.5h to 4h when administered with food. Compared with fasting condition, Cmax and AUC increased by approximately 6-fold following a moderate meal. Based on these results, a protocol amendment to the clinical trial has been implemented in order to further investigate the administration with food in a new dose escalation.

Summary/Conclusions: S55746/BCL201 monotherapy showed first signs of activity across the tested dose levels with an acceptable safety profile so far. Based on PK food interaction results, dose escalation in the fed state has started.

E1019

INCREASED VIRUS-SPECIFIC IMMUNE RESPONSES PARALLELED BY A PNEUMOCOCCUS-SPECIFIC-IMMUNODEFICIENCY STATE AND HYPOGAMMAGLOBULINEMIA: ALREADY EMERGE IN HIGH-COUNT MONOCLONAL B LYMPHOCYTOSIS PRIOR TO CLL


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Background: Patients diagnosed with chronic lymphocytic leukemia (CLL) display a high incidence of infections, due to an associated immunodeficiency state that includes hypogammaglobulinemia. Even more, it has been recently shown that the earlier stages of disease, i.e. high-count monoclonal B lymphocytosis (MBL2), subjects also have increased risk for infection. Aim: To evaluate the status of the humoral immune response in CLL at different disease stages, as well as in pre-leukemic MBL1 and MBL low count (MBLb) cases, vs healthy controls, through quantitation of soluble plasma levels of specific antibodies against ubiquitous and pulmonary infection-associated pathogens.

Methods: A total of 249 subjects (119 males/130 females; aged 68±11y) including 91 healthy donors, 71 CLL-like MBL1b, 29 CLL-like MBL1b 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 isotypes and specific immunoglobulins against CMV (cytomegalovirus), EBV (Epstein Barr Virus), influenza virus and S.pneumoniae were performed by nephelometry and commercial ELISA kits, respectively. Individuals who had received vaccination against Influenza and/or Pneumococcus were excluded from the analysis of the immunoglobulin-specific titers against the corresponding pathogen, respectively. Plasma CMV and EBV DNA load were measured using real-time PCR.

Results: Total immunoglobulin (Ig) titers tended to decrease with disease progression, independently of the isotype. In contrast, specific IgM and IgG titers against CMV, EBV and influenza virus did not vary among groups, with the
exception of VCA-EBV IgG titers, that were higher in CLL vs the other groups. Strikingly, the IgG levels for the three viruses tended to gradually increase, from healthy individuals to stage B/C LL. These findings were more pronounced (p<0.05) for IgG and to a lesser extend also for IgM, when the ratios between the virus-specific IgG/total IgG titers of the same isotype were calculated, except for Influenza-specific IgG, that showed the same trend but without statistical significance. Repeat CMV DNA load, only on 3177 individuals (1 MBL and 2 CLL- were found to be positive (below the limit of quantitation), while EBV DNA load was detected in plasma from 7/191 (all been Binet A CLL) at medium levels of 3.6 copies/ul. In contrast to the virus-specific Igs, IgG plasma levels against S.pneumoniae progressively diminished through progression of the disease, leading 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 virus-specific 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 ALTERATIONS AND TP53 DISRUPTION AS PREDICTORS OF OUTCOME AND CHEMOREFRACTORINESS**

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**Background:** Chronic lymphocytic leukemia (CLL) is a heterogeneous disease, running an indolent course in some patients and a clinically aggressive course in others. Risk assessment is important in clinical practice and prediction of outcome and response to treatment is very useful in an era in which several chemomunotherapy combinations and effective mechanism-driven treatments are available.

**Aims:** We investigated whether an extended genetic characterization including mutational screening by next generation sequencing (NGS) and karyotype analysis could allow for a refinement of our capability to predict outcome in newly diagnosed CLL patients with high-risk features, as defined by the presence of unmutated IGHV gene and/or 11q22/17p13 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, ie, at least 3 chromosome aberrations); intermediate group (all other karyotypic abnormalities). A cut-off of 98% homology to the germline sequence to discriminate between IGHV mutated and unmutated cases. Mutation screening was performed on 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 (55.3%) cases; 80 nonsense mutations, 5 nonsense mutations and 10 frameshift deletions. 16 cases (15.8%) 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 22 mutations by NGS (p=0.001) and a complex karyotype (p=0.012). By multivariate analysis an advanced Binet stage (p=0.001) and an unfavorable karyotype (p=0.01) predicted for 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 (TCR) 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 TCR 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 TCCR. Since karyotyping using novel mitogens 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.
PRACTICE FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

E1023

Figure 1.

Summary/Conclusions: The current analysis provides insight into adherence patterns of patients on long-term rituximab treatment. This result is 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 provide information that allows a PSP to adapt to patients’ evolving needs over the treatment journey. Over the past few years, oral-targeted therapies have been approved in the US for CLL pts, including those positive for del(17p).

Aims: This study used an electronic health record database to characterize treatment and del(17p) testing patterns in the first 2 lines of therapy (LoT) for CLL pts initiating treatment between 2011–2016. The association between del(17p) testing and utilization of targeted therapies was also explored.

Methods: This was a retrospective observational study in CLL utilizing a large, longitudinal, demographic and geographically diverse database of US cancer pts (Flatiron Health 12/2016). An analytical cohort of pts treated at community practice sites who initiated 1st LoT after Jan 2011 was developed. Two sub-cohorts of pts who initiated 1st LoT before and after 2014 were also identified. Analyzing the approval timing of oral-targeted therapies in the US:

Results: As of Dec 2016, 3,140 pts with CLL were included in the broad Flatiron Health CLL cohort. The results of this analysis are based on the analytical cohort that included 1,700 pts who initiated 1st LoT during 2011–2016, of which 1,134 (66.7%) pts initiated therapy after 2014. Second LoT was initiated in 622 pts (36.6%) between 6/2016, with 216 of the 2nd LoT initiations occurring after 2014. Average age at CLL diagnosis was 66.9 years; 63.2% of pts were male; 70.2% were Caucasian. Overall, 2011–2016, the rate of genetic testing by cytogenetics or fluorescence in situ hybridization (FISH) before initiation of 1st LoT was 67.4%. The rate of del(17p) specific testing before 1st LoT was 59.1% (an increase from 38.9% in 2011 to 64.4% in 2016). Among those who were tested before 1st LoT, 12.5% tested positive for del(17p). In the sub-cohort of pts who initiated 1st LoT after 2014, rituximab monotherapy replaced fludarabine, cyclophosphamide and rituximab (R) combination as the 3rd most frequent (13.0%) 1st LoT in pts with CLL, after bendamustine/rituximab (BR) combination (29.8%), and rituximab (R) monotherapy (17.7%). In contrast, ibritinib became the most common (43.9%) 1st LoT in newly diagnosed CLL pts with del(17p), followed by BR (24.5%) and R (7.1%). 36.6% of pts initiated 2nd LoT by December 2016. The three most common treatment sequences from 1st LoT to 2nd LoT were CIT to B-cell receptor pathway inhibitor containing therapy (16.4%), CIT to CIT (14.4%), and immunotherapy (IT) to IT (12.4%). Overall, the utilization of oral-targeted therapies has steadily increased since 2014, and multivariate analyses indicate that the presence of del(17p) is strongly associated (OR=8.7 and 2.8 for 1st and 2nd LoT, respectively) with this choice once these agents became available in 2014.

Summary/Conclusions: Considerable treatment pattern changes were observed for CLL pts in the US community practice due to the adoption of newly approved targeted therapies. Presence of del(17p) is strongly associated with choosing a targeted therapy regardless of LoT. Future research is needed to determine how differences in pt and disease characteristics and cytogenetic testing patterns influence treatment decisions and associated outcomes.

E1024

SINGLE-AGENT IBRUTINIB VS REAL WORLD TREATMENT FOR PATIENTS WITH TREATMENT-NAIVE (TN) CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): AN ADJUSTED COMPARISON OF RESONATE-2™ WITH THE CLLEAR AND LYON-SUD DATABASES


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Background: The phase 3 RESONATE-2™ study demonstrated significant improvement of progression-free survival (PFS) and overall survival (OS) with ibritinib (ibr) vs chlorambucil (chl) in TN (aged ≥65 years) CLL patients. In the absence of direct comparison of single-agent ibr with other frequently utilized treatments in this patient population, we conducted an adjusted comparison of patient-level data from RESONATE-2™ vs RESONATE-2™.

Methods: This retrospective observational study included comparison of patient-level data from RESONATE-2™ vs RESONATE-2™. The rate of del(17p) specific testing before 1st LoT was 59.1% (an increase from 38.9% in 2011 to 64.4% in 2016). Among those who were tested before 1st LoT, 12.5% tested positive for del(17p). In the sub-cohort of pts who initiated 1st LoT after 2014, rituximab monotherapy replaced fludarabine, cyclophosphamide and rituximab (R) combination as the 3rd most frequent (13.0%) 1st LoT in pts with CLL, after bendamustine/rituximab (BR) combination (29.8%), and rituximab (R) monotherapy (17.7%). In contrast, ibritinib became the most common (43.9%) 1st LoT in newly diagnosed CLL pts with del(17p), followed by BR (24.5%) and R (7.1%). 36.6% of pts initiated 2nd LoT by December 2016. The three most common treatment sequences from 1st LoT to 2nd LoT were CIT to B-cell receptor pathway inhibitor containing therapy (16.4%), CIT to CIT (14.4%), and immunotherapy (IT) to IT (12.4%). Overall, the utilization of oral-targeted therapies has steadily increased since 2014, and multivariate analyses indicate that the presence of del(17p) is strongly associated (OR=8.7 and 2.8 for 1st and 2nd LoT, respectively) with this choice once these agents became available in 2014. Considerable treatment pattern changes were observed for CLL pts in the US community practice due to the adoption of newly approved targeted therapies. Presence of del(17p) is strongly associated with choosing a targeted therapy regardless of LoT. Future research is needed to determine how differences in pt and disease characteristics and cytogenetic testing patterns influence treatment decisions and associated outcomes.

E1023

TREATMENT AND 17P DELETION TESTING PATTERNS IN COMMUNITY PRACTICE FOR PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN THE UNITED STATES

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Background: CLL is the most common type of leukemia in adults in the US. Traditionally, chemotherapy - and chemo-immunotherapy (CIT) have been the treatment mainstay. Historically, CLL patients (pts) with high-risk genetic features (e.g., 17p deletion (del(17p))) have poor prognosis and few treatment options. The current analysis provides insight into adherence patterns of patients on long-term rituximab treatment. This result is 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.

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. Patient adherence was calculated using the Morsky Medication Adherence Scale© score, which informed nurse coaching frequency. Adherence was delineated by prescription refill compliance. Patient and physician questionnaires were used to gauge satisfaction with the YOU&i™ PSP.

Results: As of 20 January 2016, a total of 903 patients with CLL were enrolled in the YOU&i™ PSP. A total of 552 patients were included in the adherence analysis. Of these, 86% opted in to receive the nurse coaching component. At 2 months from treatment initiation, patients who received nurse coaching demonstrated an adherence rate of 92.3%, as compared with 63.5% for patients who did not receive nurse coaching (OR=17.5; 17.5-41.4; p <0.0001). At 9 months, adherence rates were 89.9% vs 60.8% (95% CI, -18.9 to 32.4; p=0.715). By 9 months, adherence rates were 81.7% vs 28.4% (95% CI, 72.2% to 32.4; p=0.0001) vs 28.4% (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.

Aims: hard.
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 RA1/IBINET), and deletion 11q pres-
ence/absence instead as covariates.

Results: Median age at treatment initiation for CLCLEAR (n=1418) 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 CLCLEAR and 57% in
Lyon-Sud vs 65% in RESONATE-2™. The median follow-up was 35.7 months
(months; mo) for Lyon-Sud and 16.8 mo in CLCLEAR vs 29.1 mo for RESONATE-2™.
Adjusted HR for ibru vs physician choice in CLCLEAR 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), Chi alone (n=43), Chi+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 both databases.

Figure 1.

Summary/Conclusions: This adjusted comparison of patient-level data from
RESONATE-2™ with RW data from CLCLEAR and Lyon-Sud demonstrates ibru
to be more effective compared with RW treatment, with a 4-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 comparis-
ones on OS. These results further support the existing evidence that ibru sig-
nificantly improves PFS and OS vs common regimens used in TN CLL settings,
and has important implications for clinical practice.

E1025

CHARACTERISTICS, TREATMENT, AND OUTCOMES OF ≥80 YEAR OLD PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) ENROLLED TO PROSPECTIVE TRIALS OF THE GERMAN CLL STUDY GROUP (GCLSG). O. Al-Sawai1,1, J. Bahlo1,1, K. Fischer1,1, C. Herlinger1,1, M. Hochstetler1,1, A. Fink1,1, J. von Tresckow1, P. Langerbeins1, P. Cramer1, S. Stilgenbauer3, C. Wendtner2, B. Eichhorst1, M. Hallek1, V. Goede1

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Background: People over 80 years are the fastest growing age group in western
populations. Clinical management of ≥80 year old patients (pts) with CLL
remains a challenge due to the very limited amount of data currently available
for this age segment. Two retrospective studies reported observational data on
characteristics, treatment, and outcomes of ≥80 year old pts not enrolled in a
clinical trial (Bahlo et al., Meunier et al.). There is little known about ≥80
year old pts who were treated for CLL within clinical trials, however.

Aims: To study the characteristics, treatment, and outcomes of pts aged ≥80
years who received their first therapy within prospective trials of the German
CLL Study Group (GCLSG).

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 ≥80 years at frontline treatment. Clinical, laboratory, and genetic data of
identified pts were pooled. Time-to-event data were analysed by Kaplan-Meier
methodology. Independent prognostic factors for survival were identified by
multivariate analysis using Cox regression modelling with stepwise selection
procedures.

Results: Among 3552 reviewed GCLLSG trial participants, 152 were aged ≥
80 years at initiation of firstline treatment. A majority of these pts were identified
from CLL1 (n=132) while the remaining were from CLL1 (n=3), 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 cre-
tinine clearance was 46 ml/min (range 17-99 ml/min). Identified genomic
aberrations were 13q deletion as a sole abnormality in 27%, trisomy 12 in 18%,
11q deletion in 9%, and 17p deletion in 16% of pts. (IGH) was unmutated in
69% of the pts. Distribution of CLL-IPI risk groups was as follows: 6% low, 19%
intermediate, 61% high, and 14% very high. Most pts had Binet Stage B (36%)
 or C (43%). Chemoimmunomotherapy with chlorambucil plus obinutuzumab (CL-
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, F=n1), fludar-
bine/cyclophosphamide/rituximab (FCR, F=n2), 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.

Summary/Conclusions: Findings suggest that antileukemic therapy (including
chemoimmunotherapy) is feasible and efficacious in ≥80 year old pts with CLL.
However, such pts are still highly underrepresented in clinical trials and even
with modern treatment live shorter than age-matched controls of the general
population. Broader recruitment of these pts to prospective trials and evaluation
of targeted therapies therefore appears imperative to improve outcome of CLL
in this age segment.

E1026

THE ROLE OF CD200 IN THE DIAGNOSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA A. Mora1,2, E. P. Vicente1,2, C. Cuellar1,2, R. Bosch1,2, L. Blanco3, R. Martino3, J. M. Ubeda3, J. Sierra3, C. Moreno3, J. Normen2

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Background: Clinical, morphologic, immunophenotypic and genetic features are
the basis for the diagnosis of B-cell malignancies. It is considered that the
diagnosis of CLL requires the presence in peripheral blood of ≥5x10⁹/L monoclonal
B lymphocytes with a distinctive immunophenotype (i.e. SmIg+, CD5-, CD19,
CD23+). Based on immunophenotypic characteristics, Matutes et al de
declared in 1994 a immunophenotypic score based on a few markers (CD5+, CD23+,
FMC7+, SmIg+, and CD22+). Each one of them receiving a score of 1
if present or 0 if absent. A total score of 4 or 5 is typical of CLL whereas those
cases scoring 0 or 1 correspond to other B-cell malignancies, mostly lymph-
omas. Nevertheless, clinical and immunophenotypic features of CLL may
collapse with other B-cell malignancies. CD200 has been described as a marker
potentially useful to distinguish CLL from other CD5+B-cell malignancies.

Aims: The aim of this study was to analyze whether the addition of CD200 to
the Matutes score improves the diagnostic accuracy of CLL.

Methods: We prospectively assessed the immunophenotype of 99 peripheral
blood samples of patients with suspected lymphoproliferative disorders
between November of 2015 and January of 2017. Immunophenotyping was
performed using a Canto Flow Cytometer (Becton Dickinson) and samples
were stained with routine combinations plus CD200. The Matutes score
was calculated as follows: FMC7, CD22 and CD79b were considered score 1
in patients with ≥80% cells positive. CD22 and CD23 were considered score
1 when the positive cells were >30%. The cut-off used for CD200 was
calculated by Receiver Operating Characteristics (ROC). CD200 was scored
as 1 when the positive cells were >96%. Mean Fluorescence Intensity Ratio

Figure 1.

Summary/Conclusions: Findings suggest that antileukemic therapy (including
chemoimmunotherapy) is feasible and efficacious in ≥80 year old pts with CLL.
However, such pts are still highly underrepresented in clinical trials and even
with modern treatment live shorter than age-matched controls of the general
population. Broader recruitment of these pts to prospective trials and evaluation
of targeted therapies therefore appears imperative to improve outcome of CLL
in this age segment.
(MFI) was calculated as a relative expression between MFI positive population and MFI negative population. Multivariate analysis was used to assess statistical significant differences in accuracy among individual markers and scoring systems. The treating physician made the final diagnosis of the different B-cell malignancies according to IWCLL and WHO criteria. Logistic regression including sensitivity, specificity and accuracy values, were used to evaluate statistical differences in diagnostic precision between different combinations of markers as well as individual markers.

**Results:** Flow cytometry analysis was performed in 99 patients, including 62 cases with a diagnosis of CLL (82.6%) and 37 cases with a “non-CLL” diagnosis (37.4%). Matutes score was 4-5 in all CLL cases and ≤3 in “non-CLL” cases. CD20, CD23 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 relevant markers were SmIg, FCMI7 and also CD20. The analysis of the accuracy is shown in the table. Of note, CD200 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 CD200 did not vary when comparing% of positive cells with MFI. In contrast, the accuracy for SmIg significantly increased from 67.7% to 78.8% when using MFI 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.**

<table>
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<th>Marker</th>
<th>Score punctuation</th>
<th><strong>CD20+ Non-CLL (%)</strong></th>
<th><strong>CD20+ CLL (%)</strong></th>
<th><strong>p-value</strong></th>
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<td></td>
<td>positive</td>
<td>62.3</td>
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**Summary/Conclusions:** These results confirm CD200 as a valuable marker in the diagnosis of CLL.

**E1027**

**COMPARISON OF CHROMOSOME BANDING ANALYSIS AND GENOMIC MICROARRAY TECHNIQUES FOR THE DETECTION OF COMPLEX KARYOTYPES IN CHRONIC LYMPHOCYTIC LEUKEMIA**


**Background:** Well-established poor prognostic factors in chronic lymphocytic leukemia (CLL) include cytogenetic abnormalities (≥3 abnormalities, CK) by CBA were included [Median age: 73; 15 males (63%)]. Median time from diagnosis to CBA/microarray analysis was 3 months (range, 0-160), and 4 patients (16%) had received prior treatment. The cohort was enriched in del(17p) and del(11q23) (47% and 42%, respectively). DNA from peripheral blood lymphocytes was hybridized to Cytogenet whole-genome 2.7M (n=2) or CytoScan HD (n=22) array, results were analyzed with Chromosomal Analysis Suite Software (Affymetrix). Number, size and type of aberrations detected were compared between techniques.

**Results:** A median of 3.5 aberrations (range: 3-9) were detected by CBA, being significantly lower than the copy number abnormalities (CNA) identified by microarrays (median 5, range: 1-28; P=0.018). The median size of the CNA was 5.4Mb (range: 0.1-174Mb). Current recommendations for microarray analyses suggest that only CLL known abnormalities and CNA >5Mb should be considered for clinical interpretation (Schoumans et al, 2016). When applying this cutoff, 42% of the initially detected CNA (74/177) were omitted and no significant differences in the number of abnormalities by each technique were found (P=0.334). CNA <1Mb did not involve any chromosomal altered region in the corresponding karyotype. Thus, their omission probably would not affect the stratification based on complexity. In contrast, most of the CNA between 1 and 5Mb involved small CNA associated to apparently balanced translocations by CBA, and in some cases revealed a higher genomic instability than the previously recognized by CBA (i.e. multiple deletions defined as a single one or a monosomy by CBA). Indeed, four cases showed chromothripsis not detected by CBA which has been associated with impaired outcome (Salaverria et al, 2015). Of note, genomic microarrays failed to detect some balanced translocations or subclonal aberrations by CBA, which probably were represented in a minor proportion of the sample but expanded during CBA culture. Thus, eight patients (21%) could only be considered complex by CBA, as by microarray analyses <3 CNA were detected. The present study is ongoing; additional cases have been collected in order to statistically assess the clinical impact on survival of the complexity detected by microarrays.

**Summary/Conclusions:** 1. The number of chromosomal abnormalities detected in CLL patients differs if assessed by CBA or genomic microarrays. 2. The current 5Mb cut-off to define clinically relevant CNA should be revised, as it could underestimate genomic instability (contiguous small deletions, chromothripsis). 3. More studies should be performed to establish standard criteria for prognostic stratification of CLL patients based on genomic complexity consistent with the results from both techniques.

**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/or 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 Freeliteimmunoassay. The normal free kappa chains level was defined as within the range of 3.3-19.4mg/L, and the normal lambda chains level within the range of 5.71-26.30mg/L. A normal sFLC kappa/lambda(K/L) ratio was therefore defined as between 0.26 and 1.65 (a ratio above 1.65 indicating an excess of kappa light chain, and a ratio below 0.26 indicating an excess of lambda light chain). The cumulative level of kappa plus lambda (K+L) was also evaluated. Most patients received combined chemo-immunotherapy or entered clinical trials whenever possible. The ROC methodology was used to establish the best cut-off value of sFLC ratio level to discriminate treated patients from those who remained treatment-free.

**Results:** Main patients characteristics are detailed [N=147, M/F:75/72, 111 in early stage disease, Del1p in 11 patients and Del1q in 15]. Median age was 54 years.
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±41.9 respectively (p<0.0001).

Treatment systematically induced a modification of the sFLC K/L ratio. Interestingly, after treatment completion, the persistence of an abnormal sFLC K/L ratio was associated with frequent bleeding complications, explained by inhibition of BTK, which mediates downstream signaling of GPVI and GPIb receptors in platelets. Levels of CD42b, CD61, CD62P, PAC1, annexin V binding, and peptide. Levels of CD42b, CD61, CD62P, PAC1, annexin V binding, and

Methods:
Forty-three patients with relapsed and refractory CLL and 10 healthy donors were included in the study. Platelet functional activity was characterized by flow cytometry before and after activation with SFLLRN plus collagen-related peptide. Levels of CD42b, CD61, CD62P, PAC1, annexin V binding, and megacarpel release were determined. Aggregation with collagen, ADP and ristocetin were measured. All tests were performed before initiation of treatment, at weeks 2, 4, 8 and at 6 months. Bleeding complications were scored using ITP-specific Bleeding Assessment Tool.

Results:
Among 43 CLL patients, 29 (67%) were men, the median age was 65 (range 31 to 83 years). Four patients with del (17p) received ibrutinib as a first line. In 39 previously treated patients the median number of prior treatments was 3 (range, 1-6). Del17p or TP53 mutation was found in 11 (25%) patients. Only 1 patient received anticoagulant and antiplatelet drugs. Median duration of ibrutinib treatment was 8.2 months (range 2.2-10.9). At least one bleeding episode occurred in 23 patients (53%). Among patients with bleeding, 14 (61%) had grade 1 events, 7 (30%) had grade 2 and two (9%) had grade 3 events. Bleeding frequency decreased with time on ibrutinib: only 4 patients still had bleeding episodes after 6 months. The patients with bleeding had slightly lower mean platelet count that those without (120 versus 170 thousands per microliter, P<0.0001) and higher lymphocytosis (74 versus 45, P=0.05). Their activation of integrins in response to stimulation was greatly impaired (9% versus 26%, P<0.0001; while the 95% confidence interval for healthy controls 63-137%), and there was significant deficiency in procoagulant activity as well (2% versus 5%, P=0.01; normal range is 7-35%). Importantly, the integrin activation allowed risk stratification: a person with more than 9% integrin activation had less than 10% risk to develop bleeding while the one with less than 9% integrin activation had 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 assays 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 between ITP-specific bleeding assessment and integrin activity level.

Summary/Conclusions: Both classic aggregation assays and flow-cytometry-based techniques demonstrate impaired platelet function in the bleeding CLL patients compared with non-bleeding ones. The level of integrin activation appears to be the most sensitive and able to identify patients with different bleeding risks.

E1030 HYPOGAMMAGLOBULINEMIA IS A STRONG PREDICTOR OF TIME TO FIRST TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Management of chronic lymphocytic leukemia (CLL) dramatically improved since the introduction of novel therapies. Rarely patients requires treatment at diagnosis and approximately a third of patients will never require therapy. Predictive and prognostic factors are well known (IGHV, del11q, del17p, TP53); CLL-IPI score including age, clinical stage, beta2microglobulin, IGHV and deletion 17p and/or TP53 mutation has been recently validated. It identifies 4 risk groups with significantly different time to first treatment (TTFT) and overall survival (OS). Hypermobagglubulinemia (HYPO) is a typical feature of CLL, with an incidence of 20-60% at diagnosis and a relationship with infections occurrence. Prognostic significance of HYPO at diagnosis has not been extensively evaluated in terms of OS and TTFT. Only IgG serum levels have been reported to be associated with TTFT but no data are available on other immunoglobulin classes (Ig).

Aims: To evaluate the impact of HYPO and single Ig classes on TTFT in a retrospective cohort of CLL patients and to assess the relationship between HYPO and CLL-IPI.

Methods: We retrospectively evaluated 698 consecutive CLL patients diagnosed at our Institution from 1983 till 2016. Data from laboratory, biological analysis and clinical stage were collected. We also evaluated immunoglobulin (Ig) subclasses (i.e. IgG, IgM and IgA) at diagnosis and calculated CLL-IPI. HYPO was defined basing on our laboratory cut-offs (IgG 70mg/dl, IgG 700mg/dl, IgG 40mg/dl). However, as no recognized prognostic/predictive Ig cut off has been reported to date, we aimed to identify a prognostic threshold for each Ig class.

Results: From 698 patients assessed, 410 cases were evaluable for Ig levels at diagnosis. IgA levels were lower than 70mg/dl in 17.4%, IgG lower than 700mg/dl in 22.2%, and IgM lower 40mg/dl in 33.7%. Forty-six percent of patients presented deficit of at least one Ig class, while 7.8% of patients had all Ig low. Each Ig deficit was related with a shorter TTFT with the following hazard ratios (HRs): 2.09 (1.45-3.03) for IgA (P<0.0001), 1.58 (1.10-2.27) for IgG (P=0.008) and 1.52 (1.09-2.13) for IgM (P=0.01) (Figure 1, A-B-C). However, only IgA deficit maintains statistical significance in multivariate analysis [HR 1.59 (1.08-2.35)]. A prognostic threshold for each Ig class was identified maximizing the differences in TTFT and the following values were obtained: 80mg/dl for IgA, 410mg/dl for IgG and 18mg/dl for IgM (Figure 1, D-E-F). Considering CLL-IPI, 18 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 IgG values, when using the newly validated Ig cut-off. Finally, CLL-IPI was a stronger prognostic factor for TTFT than HYPO in our analysis. However, the addition of IgA deficit to CLL-IPI appears to further improve CLL prognostication.

Figure 1.

Summary/Conclusions: In conclusion, HYPO significantly impacts on CLL prognosis. Moreover, even if CLL-IPI has a stronger prognostic value for TTFT compared to HYPO, the addition of IgA deficit appears to further improve CLL prognostication.

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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 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 or 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 higher ALC (p<0.001), higher B2M (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=0.001), 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).

Conclusion: A short LDT is a relevant parameter for OS. Further studies should address whether this result also applies to patients treated with novel agents.

Figure 1.

Summary/Conclusions: All IFs are still regarded as equally important, although no solid evidence exists to support such statement. In our series, infiltrative cytopenia and/or progressive lymphadenopathy/splenomegaly constituted the IT in most (85%) CLL patients. In spite of being enriched in favorable biological prognostic factors (mutated IGHV genes, low ZAP70 expression and favorable-risk cytogenetics), MF patients had a shorter age-adjusted OS from first-line therapy compared to LML patients. Further studies should address whether this result also applies to patients treated with novel agents.

E1032

INDICATIONS FOR TREATMENT IN CHRONIC LYMPHOCYTIC LEUKEMIA: CLINICO-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT

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Background: Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of monoclonal CD5+ B cells in the bone marrow and lymphoid tissues. International guidelines recommend initiation of treatment only in case of infiltrative cytopenia, progressive splenomegaly or lymphadenopathy, short lymphocyte doubling time (LDT), B symptoms and/or refractory disease (IC). These criteria are based on experts' consensus and considered equally relevant for treatment initiation, even though little evidence exists concerning the relative value of each individual criterion.

Aims: To describe the clinico-biological characteristics and prognosis of CLL patients according to the criteria that prompted the initiation of first-line treatment.

Methods: Data were collected consecutively on 530 consecutive patients with CLL who received frontline therapy from 1978 to 2014 and had their indication(s) for treatment (ITT) recorded. Massive/progressive lymphadenopathy and massive/progressive splenomegaly were grouped together as lymphoid mass (LM), infiltrative anemia and thrombocytopenia were categorized as marrow failure (MF). As 505 patients initiated therapy with both ITT, we decided to focus on these two groups. Patients whose ITT was both LM and MF were classified as MF following the logic behind Rai and Binet staging systems.

Results: Median age of the whole cohort was 62 years (range 22-93), and 63% of patients were male. Median follow-up from first-line therapy was 64 months (range, 1-433). Half of the patients had a single ITT, while the other half had two or more. ITT were LM in 72% patients, MF in 31%, short LDT in 29%, B symptoms in 19%, and IC in 3%. Compared to patients from the LM group, patients from the MF group were significantly older, had a significantly higher β2-microglobulin level (probably due to an age-related impaired renal function), and were more frequently treated due to LM and had more frequent adverse prognostic characteristics, such as higher ZAP70 expression, unmutated IGHV genes, and 11q deletion. The median OS of the entire population was 77 months (95% confidence interval [CI]=71-83) from first-line therapy and 108 months (95% CI=102-118) from diagnosis. Indication for treatment was significantly associated (p=0.001) with a shorter OS from first-line therapy: 63 months in the MF group (95% CI: 48-72), compared to 89 months in the LM group (95% CI: 80-106). This association remained significant after adjusting for age and β2-microglobulin concentration.

Background: The presence of TP53-inactivation in chronic lymphocytic leukemic lymphoma (CLL), namely through the deletion of all or part of the chromosome 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 (IC), 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).
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 6-year study period. The majority of patients (63.2%) were male. Although FACS separation of neoplastic cells was only introduced within the last two years of the study period, it accounted for 39.0% of all tested samples. This poor prognostic aberration was identified in 15.8% of patients in the overall cohort, with no differences in the incidence of a positive finding between mixed cellularity samples and FACS purified samples (15.6% vs 16.2%, respectively, p=NS). In contrast, the average proportion of positive cells within a positive sample was markedly different between mixed cellularity samples and FACS-processed samples, increasing nearly three-fold through the purification of the sample, from 24.0±15.9% to 62.9±33.3%, p<0.001. In fact, in 57.7% of all patients who were tested after FACS separation of CLL cells, the TP53-deleted clone was larger than 50% of neoplastic clonal lymphocytes, making it the primary clone.

Summary/Conclusions: We observed that the pre-processing of the sample through the FACS-supported purification of CLL neoplastic lymphocytes revealed that the TP53-deleted clone was nearly three-fold larger than suggested by the mixed cellularity sample, increasing from an average of a quarter of all cells, to nearly two-thirds. This finding uncovered that the TP53-clone was, in fact, the primary major clone within the neoplastic lymphocyte population in the majority of patients. Considering the poor prognosis conferred by the aberration, and its impact on current treatment decisions, it is quite significant to correctly identify a primary deletion-positive clone, instead of mislabeling it as a secondary minor clone.

E1034
PRIMARY PEGFILGRASTIM PROPHYLAXIS VERSUS FILGRASTIM GIVEN "ON DEMAND" FOR CLADRIBINE-INDUCED NEUTROPHILIA IN HAIRY CELL LEUKEMIA


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Background: Major advances in the treatment of patients with HCL were made in the 1980’s after the introduction of two purine analogues: pentostatin and cladribine. Both these agents dramatically altered the clinical course and outcome of this disease and induced high response rates of 75-90%, with durable remissions and subsequent median relapse-free survival of up to 15 years. The major significant short-term toxicity of therapy with cladribine are neutropenia and neutropenic fever (NF). Based on the script data: 71% of patients experienced grade 4 neutropenia (absolute neutrophil count [ANC] <500x10^9/L), and 42% develop NF. The latter complications may result in life-threatening infections, as well as hospitalization.

Aims: In this retrospective study, we compared the incidence and duration of neutropenia, NF and hospitalization in patients with HCL treated with cladribine following pegfilgrasim as primary prophylaxis versus daily filgrastim given "on demand" according to the neutrophil count.

Methods: The study population included 202 patients with HCL, diagnosed and followed in 12 medical centers in Israel during 1985-2015. Patients were treated with cladribine, for 5-7 days given either sub-cutaneously or via intravenous infusion. Medical records were evaluated for details of disease at diagnosis, including date of diagnosis, age, sex, ethnicity, complete blood count results, and spleen size at diagnosis. The efficacy of pegfilgrastim and filgrastim was assessed by evaluating the incidence of neutropenia (defined as ANC <1000x10^9/L), number and length of hospitalizations due to NF, severity of infections and the number of days from the last day of therapy until recovery of ANC to >1000x10^9/L.

Results: Mean follow up was 7.5 years (0.1-40), with 5 and 10 years’ survival of 96% and 90.62% respectively. The median age at diagnosis was 53 years, and 81.8% were males. First line therapy with cladribine was given to 159 patients, and of these 50.3% required hospitalization for the administration of broad-spectrum antibiotics due to NF. The risk factor to develop NF was WBC<0.6 10^9/L, and ANC<0.310^9/L. Twenty eight patients were treated with pegfilgrastim as primary prophylaxis 24 hours after the last day of therapy with cladribine, while 75 patients received filgrastim “on demand” due to neutropenia. Median hospitalization days, and Nadir duration was 8 and 18 days respectively in both groups (p=0.71, p=0.44).

Table 1.

Summary/Conclusions: Infectious complications post cladribine treatment, remains high, with an incidence of 50.3%. For all parameters analyzed, including the percentage of febrile patients, number of febrile days, and NADIR duration the results of primary pegfilgrastim prophylaxis and filgrastim given on demand were similar. Accordingly, we conclude that it remains the treating physician’s choice to decide on which type of filgrastim to use and when to administer it.

E1035
REDUCED HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA ACHIEVING COMPLETE REMISSION TO FIRST-LINE THERAPY

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Background: Most targeted therapies in the management of chronic lymphocytic leukemia (CLL) lead to high overall response rates but complete remissions are rare. Achieving complete remission (CR) is associated with improved clinical outcomes such as longer time to progression; however little is known about the economic benefits associated with achieving CR.

Aims: The objective of the study was to compare healthcare resource utilization among CLL patients initiated on first-line treatment who achieved CR versus those who did not.

Methods: This was a retrospective chart review study. From July to August 2016, 93 US oncologists/hematologists provided data abstracted from medical charts of their CLL patients who initiated a first-line CLL treatment between January 2010 and December 2014. The study collected patient demographics, clinical characteristics, response to first-line therapy, and the number of all-cause hospitalizations between first-line therapy initiation and end of the data follow-up (i.e., patient’s date of death, end of care, or data collection date, whichever occurred first). Patients were selected based on their best response to first-line therapy (i.e., CR, partial remission [PR], stable disease [SD] and progressive disease [PD]) as defined by the physician according to iwCLL 2008 criteria. The targeted number of patients in each category was a priori determined based on rates of response observed in clinical trials. The incidence of all-cause hospitalization was compared between patients who achieved CR and those who did not (including patients with PR, SD or PD) using univariate and multivariate generalized linear models with a Poisson distribution. As patients had different follow-up, incidence rates were reported per-patient-per-month (PPP). Multivariate regression models were adjusted for age, gender, selected comorbid conditions, time from CLL diagnosis to first-line initiation, and Eastern Cooperative Oncology Group (ECOG) status.

Table 1.

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 cofounders, the incidence of all-cause hospitalization was 2.4 times higher for patients who did not achieve CR compared to those who did (IRR=6.4, p<0.05).

Summary/Conclusions: Results from this study showed that achieving CR to first-line therapy (vs. not achieving CR) is associated with reduced frequency of all-cause hospitalizations. This suggests that, in addition to the clinical benefit associated with CR achievement, treatment strategies in CLL that improve CR may help reducing the economic burden of CLL management for both patients and payers.

E1036
RITUXIMAB (R) USED AS A SINGLE AGENT FOR AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA) IN TREATMENT NAÏVE CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS INDUCES ALSO SIGNIFICANT DISEASE RESPONSE WITHOUT TOXICITY
E1036
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Background: There are very few effective treatment options for steroid refractory AIHA of CLL or for CLL patients(pts) that are unable to receive corticosteroids. R has been noted to be active in certain autoimmune hematologic disorders while experience with single-agent R in untreated CLL pts is very limited. Aims: To report our experience concerning the use of R as a treatment of AIHA occurring during the clinical course of treatment naïve CLL pts, by analyzing concomitantly its efficacy and safety as a single agent in CLL therapy. Methods: 15 pts diagnosed with CLL who received R due to AIHA were included in this study. Staging was performed at diagnosis (Binet system). Pts were placed on R at the standard dose I.V of 375mg/m2 once weekly for 6 consecutive weeks because of contraindication of corticosteroids administration. Results: Pts’ median age was 60 (range, 42-83 y). (8 out of 15, males), 10 having disease stage A and 5 B. Two were presented with splenomegaly and 1 with B-symptoms. 12 pts (83%) had leukemic lymphocyte counts of more than 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 course (1-15 pts) 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 splenomegalic pts. After a median follow up of 84, 5 mos from CLL diagnosis, 14 pts are alive, 9 maintain their disease response, while 5 were in need of therapy due to CLL progression, after a median time of 10 mos from the last R infusion. Among them 4 were placed on FCR (2CR, 2PR) and 1 on R-Bendamustine(PR). Median PFS has not reached. All pts received the entire first dose on day 1 of treatment. There was only a grade 3 infusion related reaction in a pt with WBC=400x10^9/L without need for hospitalization. None of the pts experienced severe tumor lysis syndrome, pulmonary insufficiency, myelosuppression or opportunistic infections.

Table 1.

<table>
<thead>
<tr>
<th>WBCx10^9/L</th>
<th>n</th>
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<tr>
<td>63 (69, 541.4)</td>
<td>6</td>
</tr>
<tr>
<td>67 (69, 541.4)</td>
<td>6</td>
</tr>
<tr>
<td>67 (69, 541.4)</td>
<td>6</td>
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</table>

Summary/Conclusions: A) R is an effective agent for AIHA treatment with concomitant significant activity against CLL and therefore could be the standard of care for CLL pts with AIHA, especially for the cohort of pts with comorbidities. B) We confirm previous data that: 1) single-agent R induces significant 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/w/o minimal residual disease in first-line chemoimmunotherapy has been associated with more favorable progression-free survival (PFS) and overall survival (OS). However, patients (pts) with R/R CLL and/or those with TP53 abnormalities (ie, 1p7 deletion and/or TP53 mutation) are less likely to achieve deep responses and experience poorer outcomes. Therefore, less is known about the relationship between CR and survival outcomes in R/R CLL pts.

Aims: To quantify this association, we generated meta-analytic estimates of PFS and OS reported in clinical trials using the proportion of study patients with CR as a predictor variable.

Methods: We performed a systematic literature review of PubMed/EMBASE up to Nov 2014 and congress abstracts 2012–2014. Randomized controlled trials and observational studies evaluating any treatment in R/R CLL pts were eligible for inclusion. Data were extracted from publications as median survival, the proportions of pts surviving at specific follow-up times, or individual event occurrence data. Kaplan-Meier (KM) curves, along with the proportion of pts with CR. Data were synthesized to estimate overall OS and PFS including population-level CR as a covariate using a Weibull proportional hazards model within a Bayesian meta-analysis framework.

Results: 74 published studies of treatment outcomes in R/R CLL pts were identified from the peer-reviewed literature and congress abstracts. 56 of these studies reported the proportion of CRs together with either OS or PFS outcomes and were included in the analysis. Individual pt data were extracted from KM curves of 29 studies generating 5176 individual pt OS and PFS data points in addition to 54 study-level data points including 3638 pts. There were no clinically meaningful differences in study or pt characteristics among the included studies that were not also associated with CR, our variable of interest. The hazard ratio (HR; and 95% credible interval, the Bayesian analog to confidence intervals) of survival for each 10% increase in CR among a study population was estimated to be 0.64 (0.60, 0.68). Estimated median OS for hypothesized conditions with 0% CR, 25% CR, or 50% CR were 20.4 mo, 44.7 mo, and 61.9 mo. Corresponding median PFS estimates were 10.0 mo, 21.9 mo, and 30.3 mo. (Figure 1).

Figure 1. Weibull meta-analytic estimates of OS (A) and PFS (B) with median survival times for a population with 0% CR, 25% CR, and 50% CR.
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 institute. The final analysis has been limited to the 486 cases with complete data to apply the MDACC score, and to the 258 cases with complete data to apply the CLL-IPI score.

Results: Median age was 67 years old (25-90). With a median follow-up time of 46 months, 394 patients were alive, and 187 had received any treatment for CLL at the moment of the analysis. Median overall survival (OS) of the whole series was 173 months (127-220), and median time to first treatment (TTFT) 106 months (82-130). The MDACC score was applied to 486 cases giving 0 to 9 points to each case according to: age, b2-microglobulin levels, absolute lymphocyte count, sex, Rai stage, and number of involved lymph node groups. As shown in the Table, stratification of patients using the MDACC score allowed the prediction of progression for both TTFT (P=0.000) and OS (P=0.000). 162 patients were classified as low risk, 302 as intermediate risk, and 21 as high risk. Due to missing data, the CLL-IPI score could only be applied to 258 patients giving 0 to 10 points to each case according to 17p deletion, IGHV mutational status, 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.

Summary/Conclusions: In this study we confirm that both scoring systems are also easily applicable in clinical practice. The new CLL-IPI score is able to discriminate patients in different prognosis subgroups. Both scores are able to provide prognostic information in these patients.

Table 1.

<table>
<thead>
<tr>
<th>Score</th>
<th>OS (P-value)</th>
<th>TTFT (P-value)</th>
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<tbody>
<tr>
<td>MDACC</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>CLL-IPI</td>
<td>0.000</td>
<td>0.000</td>
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</table>

E1038

AN OBSERVATIONAL STUDY EVALUATING THE USE OF BENDAMUSTINE AS FIRST-LINE TREATMENT FOR CHRONIC LYMPHOCYTIC LEUKEMIA IN RUSSIA

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1Hospital Universitario Infanta Leonor, MADRID, 2IBSAL, IBMCC, Centro de Investigación del Cáncer, Universidad de Salamanca-CSIC, Salamanca, Spain

Background: Bendamustine has gained footing as a component of first-line therapy for chronic lymphocytic leukemia (CLL) due to its efficacy and favorable toxicity/haematological profile. There is a paucity of data on the effectiveness of bendamustine as first-line therapy in the Russian CLL patient population, which is needed to support drug cost reimbursement processes.

Aims: Evaluate effectiveness of first-line therapy with bendamustine for CLL in the Russian Federation.

Methods: This was a prospective, multicenter, observational study (NCT02110394) in adults (>18 yr) diagnosed with CLL who were receiving, or were scheduled to receive, first-line therapy with bendamustine plus rituximab. Patients who had prior CLL treatment (eg, chemotherapy, radiation) or had contraindications to bendamustine were excluded. Each subject was required to have 2 to 8 study visits, where Visits 2-6 reflected treatment cycles; total number of treatment cycles for each patient was determined by the study investigator. Interim and final evaluations were performed after 3 and 6 treatment cycles, respectively. Primary endpoints were overall response rate (ORR, patients achieving a complete remission or partial remission [PR]) and progression-free survival (PFS). Secondary endpoints were time to therapeutic failure, time to progression, progression-free survival (PFS), relapse or death after CR or PR, quality-of-life (EQ-5D questionnaire), and frequency of adverse drug reactions (ADRs).

Results: Of the 196 patients who enrolled between June 2012 and August 2015, 142 patients received randomized therapy (Supplementary Table 1). Of these, 139 were included in the Full Analysis Set (FAS; at least one dose of study drug) and 149 were included 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 yr. Overall, 35.6% of patients were ≥65 yr old and 80.5% had ≥1 comorbidity such as hypertension. The ORR was 63.2%; 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 cases.
patients (27.4%). Overall, 80.3% of FAS patients did not experience therapeutic failure and 85.9% did not experience disease progression during the 2-year observation period. By the end of the study, median PFS had not been reached; 2-year PFS rate was estimated as 85.9%. Improvements from baseline were observed after 6 cycles of treatment across all EQ-5D domains. No relapses or deaths occurred in the FAS; however, 2 subjects in the Safety Population experienced fatal serious ADRs (myocardial infarction \( n=1 \); acute pneumonia, infections and toxic shock, and atrial fibrillation \( n=1 \)). In concurrence with the Phase 3 trial results, hematologic disorders (19.9%; anemia, neutropenia, thrombocytopenia), most of which were Grade 2 in severity, were the most common ADRs (Safety Population; Table 1).

Table 1. Hematologic ADRs by CTCAE Grade.

<table>
<thead>
<tr>
<th>Hematologic ADR</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
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<tbody>
<tr>
<td>Anemia</td>
<td>44.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>21.7</td>
<td>17.5</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>5.3</td>
<td>0.5</td>
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</table>

Summary/Conclusions: First-line therapy with bendamustine plus rituximab was well tolerated in this Russian CLL population, including elderly patients and patients with renal dysfunction or other comorbidities. Additionally, combination therapy resulted in high rates of treatment response in the CLL. These data confirm the value of bendamustine as a first-line agent for CLL in routine clinical practice in Russia.

### Chronic myeloid leukemia - Biology

**E1041**

**MUTAGENESIS OF BCR-ABL1 IS REQUIRED FOR RESISTANCE DEVELOPMENT IN DE NOVO CHRONIC MYELOID LEUKEMIA KCL-22 CELLS BUT NOT IN RELAPSED KCL-22 CELLS EXPRESSING BCR-ABL1 INDEPENDENT RESISTANCE**

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**Background:** BCR-ABL1 kinase domain (KD) mutations are an important mechanism of resistance of chronic myeloid leukemia (CML) patients developing during the tyrosine kinase inhibitors (TKI) treatment. However, mechanisms underlying KD mutation acquisition in TKI-resistant CML cells are not yet well understood.

**Aims:** We studied an acquisition of mutations in the KD after an exposure of *de novo* and relapsed (grown in optimal growing medium for 24 months) KCL-22 cells to imatinib (IM). In addition, we examined kinetics of mutated subclones in established IM-resistant KCL-22R culture after dose-reduction of IM. We also studied changes in the expression profile of KCL-22 cultures early after exposure to IM.

**Methods:** The occurrence and kinetics of expansion of *BCR-ABL1* mutant subclones were studied using next-generation deep sequencing in KCL-22 cells treated with 0.4 µM IM and in established IM-resistant KCL-22R cells at 4 µM IM. In other sets 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 G2M-checkpoint protein GADD45A whose deficiency is associated with mutagenesis (Hollander 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 mutantclone switch from T315I to E255K in KCL-22R was accelerated after IM reduction from 4 µM to 1 or 2 µM. Moreover, the emerging of E255K sub-clones was accompanied by rapid decrease of CD38 expression in KCL-22R cells. Profiling of transitional KCL-22R culture, carrying both T315I and E255K sub-clones, revealed that T315I transcripts were expressed only in the CD38+ subpopulation, while E255K was detected only in CD38- cells. Unlike to *de novo* KCL-22 cells, BCR-ABL1 mutations were repeatedly not detected in relapsed KCL-22 cells up to 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, 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 on *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 kinase inhibitor.

Supported by the project no. 00023736 and AZV 15-31540A of MZCR and ERDF OPPK CZ.2.16/3.1.00/28007.

**E1042**

**FLOW-CYTOMETRY DETECTION OF CD26+ LEUKEMIA STEM CELLS IN PERIPHERAL BLOOD: A SIMPLE AND RAPID NEW DIAGNOSTIC TOOL FOR CHRONIC MYELOID LEUKEMIA**

D. Raspado1, S. Siriani1, L. Aprile1,2, E. Abruzzese2, A. Iurlo3, A. Sicuranza1, S. Galimberti4, L. Schiattone1, A. Gozzini5, P. Pregno6, G. Caocci7, G. Papini1, I. Ferrigino1, B. Mecacci1, M. Bocchiccia
Background: Diagnosis of Chronic Myeloid Leukemia (CML) implies documenting in bone marrow (BM) or in peripheral blood (PB) Philadelphia (Ph) chromosome by cytogenetics, molecular BCR-ABL1 fusion by FISH or BCR-ABL1 rearrangement by RT-PCR. In clinical practice, at the earliest, 24-72 hrs are needed to confirm CML by any of these assays. Lately, characterization of CML leukemia stem cells (LSCs) from BM samples by CML patients (pts) showed a specific co-expression of dipetidylpeptidaseIV (CD26) within the CD34+/CD38-/Linn- stem cell fraction and CD26 displayed 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 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+/CD26+ population was investigated in PB and when possible simultaneously in BM samples using a flow-cytometry 4-color staining procedure. 2x10^6 leucocytes were incubated with BD Pharmingen CD45/500 (c.201), CD34FITC (c.581), CD38APC (c.H71T), CD26 (c.M-A261) and negative controls. Acquisition and analysis of at least 1.0x10^6 CD45+ cells were done by FACSCanto II with DIVA 8 software (BD, Biosciences). CD26+ cells were identified by sequential gate. CD45+ and CD34+ gates were performed on viable cells identified by FSC/SSC light properties and CD34+/CD38+ population was gated applying a narrow gate excluding all CD38+ cells (Fig.1).

Results: PB samples from 107 pts with myeloproliferative features were evaluated for CD26+LSCs. Leucocytes median value was 52x10^9/L (range 5-400x10^9/L). In 83/107 (77.5%) pts we showed CD34+/CD38+CD26+ LSCs in PB and in 83/83 (100%) the diagnosis of CML was confirmed by cytogenetics, FISH and RT-PCR analysis. Median value of circulating PB CD26/Lu 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 All CD26+ PB-BM matched pairs (49/53) showed superimposable results in terms of absolute number of CD26+LSCs/Lu (19.18 and 18.73 respectively) while the percentage of CD26+ cells within the CD34+/CD38- fraction appeared lower in BM than in PB samples (median 28.18 and 37.33; range 0.87-77.14, and 5.99-55.97 respectively). In 24/107 (22.5%) PB samples and in 45/53 BM samples CD26+ LSCs were not detected and none of these samples was found Ph or BCR-ABL1 positive. Pts with CD26 neg PB/BM samples were subsequently diagnosed as idiopathic Myelofibrosis (12 pts), Myelodysplastic/Myelo-proliferative disorders (7 pts) benign neutrophilia (5 pts). Of note, we additionally studied 4 PB+BM samples of 4 Ph+ acute lymphoblastic leukemia and all scored negative for CD26+LSCs.

Figure 1.

Summary/Conclusions: Flow-cytometry evaluation of PB CD34+/CD38+/CD26+LSCs is a feasible, very rapid (about 3 hrs from sample handling to results) and highly specific alternative/complementary diagnostic tool for CML. To validate these data in a larger cohort of patients we are developing a pretreated lyophilized antibody mixture (lytotube, BD Biosciences) to maximize sensitivity and to optimize standardization and working time, with the further aim to monitor stem cells minimal residual disease in CML patients.

E1043
LIPID PEROXIDATION AND INFLAMMATORY STATUS DURING TKI TREATMENT IN CHRONIC MYELOID LEUKEMIA PATIENTS: INTERIM ANALYSIS OF A PROSPECTIVE MULTICENTER STUDY

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Background: Evidences of increased cardiovascular (CV) events, mostly atherothrombotic, in Chronic Myeloid Leukemia (CML) patients (pts) treated with some Tyrosine Kinase Inhibitors (TKIs) prompted physicians to carefully evaluate CV risk factors (CVRFs) in the choice of TKI. However, the pathogenesis behind CV events during TKIs is still largely unknown and even pts without overt CVRFs incur in CV events. We retrospectively showed that an induced “inflammatory status” during nilotinib treatment, together with genetic pro-atherothrombotic predispositions may partly explain the increased incidence of CV (Bocchia, Oncotarget 2016). These data provided the rationale to start a multicentric “Prospective study of TKI induced pro-Atherothrombotic status in CML, KIARO study” (Grant support: AIRC-IT) including Chronic Phase CML pts treated with any first line approved TKI in which clinical, genetic and biochemical pro- and antiatherothrombotic 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: Pts initially evaluated 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, oxidated-LDL (oxLDL), TNFa, 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), oxLDL (237±4.99), TNFa (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 TKI treatment. No statistic significant differences were found in the expression of these variables between the 3 groups of treatment (p>0.05). Considering the genotype frequency, we confirmed in the whole cohort a correlation between basal levels of LDL, oxLDL and IL10 with the presence/absence of the detrimental G/G allele of LDL-R (H.R. 2.21, p<0.01), LOX-1 (H.R. 2.86, p<0.01) and IL10 (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 detected in the 3cohorts (p=0.079). Interestingly, IL-10 levels were significantly higher at 3 and 12 months of treatment in the imatinib and dasatinib cohort (p<0.01 respect to nilotinib p=0.094).

Summary/Conclusions: This interim analysis, although still very preliminary, suggests that in nilotinib patients the high levels of LDL and oxLDL in combination with low levels of IL10, could induce a persistent pro-inflammatory/oxidative status potentially favoring atherothrombotic events. Additional biochemical and genetic data as well as prolonged clinical observation are needed to confirm this hypothesis. Patients enrolment and monitoring is ongoing.

E1044
TRANSCRIBED ULTRACONSERVED NONCODING RNAs (t-UCRS) IN CHRONIC MYELOID LEUKEMIA: EXPRESSION PROFILES ASSOCIATED WITH MOLECULAR RESPONSE TO THERAPY WITH TYROSINE KINASE INHIBITORS

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Results: We show that PAK1 and PAK2 are frequently deregulated in cells in vivo of haematological patients, shRNA-mediated knockdown and

Methods: We investigated peripheral blood samples from 45 CML patients and 15 healthy controls. Two panels of 481 T-UCR and 752 miRNA probes were used for RT-qPCR analysis. Differential expression was evaluated using the Mann-Whitney test followed by Benjamini-Hochberg multiple testing correction.

Results: CML samples presented significantly different expression of uc.164 (p<0.01), uc.118 (p<0.01), uc.125 (p<0.01), uc.391 (p<0.01), uc.141 (p<0.01), uc.143 (p<0.05) and uc.145 (p<0.05), when compared to healthy controls. This latter T-UCR (uc.145) was associated with development and immune regulation pathways. We analysed Sokal, Hasford and EUTOS risk scores and found uc.236 (p<0.0001), uc.39 (p<0.05) and uc.7 (p<0.05) to be associated with EUTOS low risk. Concerning therapy, dasatinib was correlated with UCs for imatinib doses, uc.4 (p<0.05) and uc.3 (p<0.05) inversely correlated with 400 and 800mg daily, respectively. Molecular response in CML samples presented a signature including uc.187 (p<0.001), uc.107 (p<0.05), uc.409 (p<0.05), uc.198 (p<0.05), uc.309 (p<0.05), uc.102 (p<0.05), uc.294 (p<0.05) and uc.361 (p<0.05). Major molecular response was identified by the altered expression of uc.198 (p<0.05), uc.215 (p<0.05) and uc.210 (p<0.05). The negative regulation of T-UCRs by miRNAs, involving T-UCR:miRNA interaction, was associated with upregulated (miR-720, miR-886-3p, miR-1274a, miR-101 and miR-129) and downregulated (miR-489 and miR-1973) microRNAs.

Summary/Conclusions: In the present study, we identified T-UCRs signatures and T-UCR:miRNA pairs associated with CML, risk scores, TKI therapy and molecular response. The expanded knowledge of RNA biology in general, together with the recent interest in the mutlity of newly discovered elements such as T-UCRs, could help to improve CML therapy.

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E1046

MIRNA PROFILING OF CIRCULATING EXTRACELLULAR VESICLES IN CML PATIENTS WITH MUSCULOSKELETAL PAIN ASSOCIATED WITH DISCONTINUATION OF TYROSINE KINASE INHIBITORS

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Background: Clinical trials of TKI discontinuation are still ongoing, approximately 60% of CML patients who achieved a deep molecular response for more than 2 years maintained a major molecular response after discontinuation of imatinib. However, the long-term prognosis and/or adverse events after TKI cessation remain unclear. Recent reports showed that transient musculoskeletal pain after TKI discontinuation occurs in approximately 30% of CML patients after stopping imatinib.

Aims: Recent evidences suggest that extracellular vesicles (EVs) that contain genetic element such as DNA, RNA, and miRNA, are important mediators of intercellular communication. We therefore studied molecular study to ascertain the possible correlation between musculoskeletal pain and EV-miRNA expression.

Methods: We investigated circulating EV-miRNAs in five CML patients who did not experience musculoskeletal events and five patients with musculoskeletal pain after stopping TKIs, as well as three healthy individuals. Peripheral blood was obtained approximately 3 months after successful TKI cessation in CML patients. Exosomes were extracted by using Total Exosome Isolation Reagent (Invitrogen, Carlsbad, CA, USA) and EV-miRNA profiling was performed with a TaqMan Low-Density Array (Thermo Fisher Scientific, Carlsbad, CA, USA), as reported previously. The relative expression level of each gene was calculated by using the comparative threshold cycle (Ct) method. Synthetic spike control (ath-miR-159; Hokkaido System Science, Hokkaido, Japan) was used as an internal control for EV-miRNA. This study was approved by the institutional review board of Tokyo Medical University (no. 930 approved 24 June 2008 and no. 3052 approved 9 June 2015).

Results: Three-way analysis of variance (ANOVA) performed for healthy controls and CML patients with and without musculoskeletal pain revealed EV-miR-140-3p to be the most significant value (P=0.00778). A t-test analysis using R software identified 10 differentially expressed EV-miRNAs for CML patients with and without musculoskeletal pain: seven miRNAs were upregulated (miR-107, miR-145, miR-140-3p, miR-539, miR-495, miR-299-5p, miR-425) and three were downregulated (miR-21, miR-218, miR-523) in CML patients with musculoskeletal pain. The up-regulated EV-miR-140-3p in all CML patients decreased after release of musculoskeletal pain.

Summary/Conclusions: CML patients with increased EV-miR-140-3p achieved levels similar to those of healthy controls after relief from musculoskeletal pain and inflammatory indicators in some CML patients who stopped TKIs; however, we did not find any positive association. Although the number of CML patients in this study is too small to draw definite conclusions, further research should investigate whether upregulation of EV-miR-140-3p expression in peripheral blood is correlated with musculoskeletal events in CML patients after TKI cessation.

E1047

SOLUBLE AND MEMBRANE-BOUND RECEPTOR–LIGAND IMMUNE CHECKPOINTS AND CHRONIC MYELOID LEUKEMIA: CORRELATIONS WITH MOLECULAR RESPONSE AND TYROSINE KINASE INHIBITOR THERAPY

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Background: Blockade of immune checkpoint seems to unleash the potential of the antitumor immune response in a fashion that is transforming human cancer therapeutics. Soluble and membrane-bound receptor–ligand immune checkpoints are the most druggable forms using agonist antibodies (for co-stimulatory pathways) or antagonist antibodies (for inhibitory pathways). Although its implications in immune response during chronic myeloid leukemia therapy without consistent biochemical abnormalities is occurring in approximately 30% 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 after TKI discontinuation occurs in approximately 30% of CML patients after stopping imatinib.

Aims: This study aimed at the analysis of lymphocyte subsets expression and plasmatic levels of immune checkpoint inhibitors during tyrosine kinase inhibitor (TKI) therapy in CML and its correlation with molecular response.

Methods: Peripheral blood samples from 35 CML patients (n=55), divided according to molecular response to imatinib, dasatinib, nilotinib, bosutinib, ponatinib and Imferon-alpha 2b (IFN-α 2b) therapy, were included in this study. Multi-parametric flow cytometry was used for the analysis of the
expression of several immune checkpoint inhibitors (BTLA, GITR, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD137/4-1BB) by different T, B, NK, monocyte and dendritic cell subsets. A 14-plex panel including BTLA, GITR, HVEM, IDO, LAG-3, PD-1, PD-L1, PD-L2, TIM-3, CD28, CD80, CD137 (4-1BB), CD27, and CD152 (CTLA-4) was analyzed by xMAP technology (Luminex®).

Results: Expression of CD137 by several lymphocyte subsets and PD-1 by regulatory T cells (Tregs) and natural killer (NK) cells were found significantly altered in CML patients under TKI therapy. These associations were observed for the cell population frequency expressing the receptor, and also for density of these molecules. Increased plasmatic levels of BTLA, HVEM, PD-1, PD-L1, and CD137 were associated with good molecular response to therapy, PD-1, PD-L1, TIM-3 and CD137 were found increased in patients that achieved MR4.5.

Summary/Conclusions: Some immune checkpoint inhibitors seem to be affected by TKI therapy in CML and their cell expression and plasmatic levels correlates to molecular response. Similar observations were described for other types of cancers, including solid tumors. Soluble and membrane-bound receptor–ligand immune checkpoints could represent interesting targets for future therapeutic monitoring and for pharmacologic interventions in CML.

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E1048
TYROSINE KINASE INHIBITORS SIGNIFICANTLY CHANGE THE EXPRESSION OF POLYCOMB GENES IN CHRONIC MYELOID LEUKEMIA

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Background: It has been reported that, notwithstanding their clinical success, tyrosine kinase inhibitors (TKIs) are not able to eradicatet 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 BM11 resulting a good predictive molecular marker (Crea, 2015).

Aims: In order to better understand the role of the PcGs genes in CML patients receiving TKIs, we analyzed the expression of 86 PcGs at baseline and after 6 months of therapy.

Methods: Buffy coats obtained from peripheral blood samples of 6 patients (5 receiving imatinib and 1 dasatinib) have been used for the RNA extraction; these RNAs were used for quantifying the BCR-ABL/ABL ratio/αS, according to the European guidelines, and the expression of the chosen 86 PcGs by real-time PCR (PrimePCR pathway kit, Biorad, Milan, Italy) at diagnosis and after 6 months of treatment. Expression values were calculated using the 2DDCT method.

Results: At the sixth month of treatment, 5 patients were in optimal response and one was “warning”, according to the 2013 ELN guidelines. After therapy, 55% of the tested PcGs resulted up-regulated and 23% of them in the majority of patients, whereas 3 genes (DNMT3B, SCML2, CBX2) were down-regulated in at least half of samples. The expression of 5% of PcGs was “mixed”, up- or down-regulated in different samples. Among the up-regulated genes, some could be relevant from a biological point of view: 1) HLF, a target for RUNX1, whose low expression in acute leukemia is correlated with poor outcome; 2) PHC2, able to silence the HOX genes, overcoming the multidrug resistance in myeloid models; 3) PCGF5, that is a marker of normal hematopoiesis; 4) M0V10, that has been reported to have an anti-viral activity, increasing levels of gamma interferon. This up-regulation is particularly interesting, because concerns all assessed samples and could explain our previous observation that Torque Teno virus replication does not occur in CML patients during TKIs therapy; 5) in the only “warning” patient, the up-regulation of SIRT1 was observed: this is in line with the observation that its up-regulation increases the oncogenic ability of K562 cells in a nude model. Among the down-regulated genes, could be relevant: 1) CBX2, that binding P16/p19 promotes the cell cycle progression; its down-expression could induce apoptosis; 2) DNMT3B, whose high levels have been reported in stem cells, and whose reduction could characterize the differentiation process; 3) ZBTB16, whose reduction could be a sign of the reduced osteoblastic differentiation, one of the mechanisms responsible for the LSC preservation in the niche; 4) SMARCA1, it too correlated to the cell cycle progression. Finally, BM11 levels resulted unmodified in 3 cases and increased in other 3.

Summary/Conclusions: We demonstrated that PcGs de-regulation occurs in CML patients during the treatment with TKIs, with possible pathogenetic implications. Huger series of patients will improve the biological suggestions coming from these preliminary data.

E1049
IDENTIFICATION OF PROGNOSTIC AND SUSCEPTIBILITY MARKERS IN CHRONIC MYELOID LEUKEMIA USING NEXT GENERATION SEQUENCING

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Background: Chronic Myeloid Leukemia (CML) is 20% of all leukemias diagnosed every year. Discovery of Imatinib Mesylate has brought a paradigm shift in treatment of Chronic Myeloid Leukemia, despite 15% - 20% patient showing resistance to this TKI. Therefore, it is important to identify susceptibility and prognosis markers, which can help us in predicting occurrence and prognosis of CML. We did Clinical Exome Sequencing, a panel of more than 4800 clinically important genes, in CML patients

Aims: To identify prognostic and susceptibility genetic markers in CML

Methods: Enrolled CML patients (n=18) were segregated as responders (n=10) and failures (n=8) as per ELN, 2013 guidelines. Healthy controls (n=5) were also enrolled. DNA from blood of subjects was subjected Next Generation Sequencing (NGS). Mutations present in one patient group and absent in opposite group were considered as prognostic markers, whereas rare mutations, present in more than 50% of enrolled patients and absent in healthy controls, were considered as susceptibility markers

Results: We discovered mutations in genes associated with cancer or cancer related functions in different patient groups as markers. Five of them: rs116201358, rs17882014, rs4014596, rs52897880 and rs2274329 in C8A, HLA-DRB1, UNC93B1, APOH and CA6 genes respectively, were present in responders; rs4945 in MFGE8 was present in failure. Mutations in HLA-DRB1 (rs17787895, rs11554462, c.239C>G), HLA-DRB5 (rs137863146), RPHN2 (rs193179333), CYP2F1 (rs116958558), KCNJ12 (rs76684759), FUT3 (rs151218854), BM10 (rs28370522) and PRSS1 (rs144422014) were present in half or more patients

Summary/Conclusions: We discovered potential genetic markers, which can help in predicting response to IM as frontline therapy. Susceptibility markers can be used as panel for to configure individuals prone to CML

E1050
FEATURES OF THE A2455G POLYMORPHISM OF GENE CYP 1A1 IN PATIENTS WITH CML

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Background: Chronic myeloid leukemia (CML) is the most common myelo-proliferative disorder characterized by the reciprocal translocation t(9;22) (q34; q11), leading to the formation of chimeric oncogene BCR-ABL on the 22q-chromosome. It is known that the protein products of the genes of cytochromes ensure homeostasis at the cellular and tissue level, carrying out the metabolism of toxic compounds that can damage the genome of the cells. Present study evaluated the role of functional polymorphisms in genes of a variety of neoplastic diseases, including leukemia. In individuals with weakened functional genotypes of A2455G polymorphism of CYP 1A1 gene expression of this enzyme and, consequently, inactivation of xenobiotic must occur very slowly, thus creating conditions for adverse action of harmful metabolites in the genome of the cells. Currently, the scientific literature discusses the role of the negative allele A2455G polymorphism of CYP 1A1 gene in the development of hematological malignancies. However, the adverse roles of genotypic variants for this gene in oncogenesis of BCR-ABL-positive patients with CML have studied not enough.

Aims: Evaluation the role of A2452G 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 "AmpliTaq® "Leucosis quantitetm M-RTT” (InterLabServices, Russia). Testing A2452 polymorphism of CYP1A1 gene was performed on a programmable thermal cycler of the company "Applied Biosystems" (USA) using test systems company "Life" (Russia) according to the manufacturer's instructions. Statistical analysis of results was carried out using the statistical software package "2009 OpenEpi, Version 2.3”.

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 were as follows: 21.9% and 1.4% - in CML patients, and 86.6%, 13.4% and 0.0% - in the control group. Observed frequencies of genotypes in the studied groups was consistent with the theoretically expected and were in equilibrium with Hardy-Weinberg equilibrium (P>0.05). There was a statistically significant decrease in carriage of the adverse alleles in the population sample comparison group patients (1.4% vs 6.7%, respectively; χ²=6.8; P=0.01; OR=2.0; 95% CI 1.17-3.282). Also detected significant association of heterozygous genotype A/G in patients with CML, compared with the control group (21.9% vs 13.4%, respectively). The risk of mutant formation of the tumor clone in carriers of this genotype was 1.8 times significantly higher compared with patients not having it (χ²=4.6; P=0.03; OR=1.8; 95% CI 1.046-3.166). The homozygous genotype A/A was found with high frequency in a population of CML patients (1.4% vs 0.0%), but due to the small number of carriers of this genotype differences in the results did not reach statistical significance (P>0.05). Functionally favorable genotype A/A was found with high frequency in a population-based sample of 86.6% vs 76.7% cases of patients. At the same time, the differences reached the threshold level of significance (χ²=2.6; P=0.01; OR=0.5; 95% CI 0.29-0.88), that is evidence of a favorable protective effect of this genotype against the development of CML.

Summary/Conclusions: Our results suggest that the G allele and the heterozygous genotype A/G A2452G polymorphism of CYP 1A1 gene are important markers of increased risk in formation of malignant tumor cells and development of CML in Uzbekistan (P<0.05). In this case, homozygous genotype A/A of A2452G polymorphism of CYP 1A1 gene has a protective character in relation to risk of CML.
in 1st line, 114 in 2nd, 56 in 3rd) only 13 had hematologic toxicity and 6 had to switch, 14 had pleural effusion grade III-IV and 9 had to switch. From 115 patients treated with nilotinib (49 in 1st line and 66 in 2nd) only 10 had hematologic toxicity and 10 switched treatment. Survival: Estimated survival by 10 years was 80%. Variables associated with survival: In the univariate survival analyses (log rank test) either from diagnosis, first therapy or first TKIs, the Sokal, Euts, Euro and EUTOS LT scores, as well as age over 70y were the only statistically significant variables associated with survival. Hematologic toxicity grade III-IV was associated with lower PFS or OS (figure 1). In the multivariate analysis (Cox model), only hematologic toxicity grade III-IV and age over 70y were independent variables.

Summary/Conclusions: 1. These results show that the probability of survival by 10 years is roughly 80%, and extend the findings of our previous work showing that this probability is not different across different sequential treatments (imatinib 1st line or post-IFN, or switched to 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. Hochhaus1,*, E. Jabbar1, H. Kantarjian2, F. Guhil3, V. Kota4, T. P. Hughes5, S. Shelet6, L. Li6, J.E. Cortes2
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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: Treatmen-naive patients with CML-CP in DASISION (NCT0481247) 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 treatment (excluding interruptions) was 54 months (range: 3-70) for patients who had a DAS dose reduction and 57 months (range: 2-71) for patients who had an IM dose reduction. Changes in level of response were tracked for patients who achieved complete cytogenetic response (CCyR) or major molecular response (MMR) before or after the first dose reduction (table). Many patients maintained or increased to CCyR or MMR following dose reductions for any AE. Hematological toxicity (9%) was the most common AE resulting in dose reduction for IM, and pleural effusion (12%) was the most common for DAS.

Summary/Conclusions: Reducing DAS doses to 80mg or 50mg was a safe and effective means of managing patients who experienced AEs in this 5-year retrospective analysis of DASISION. These results were consistent with previous reports and continued to show that efficacy was not affected by dose reductions for any cause, including pleural effusion. Notably, there was no loss of CCyR following dasatinib dose reductions. Molecular responses remained higher for DAS vs IM irrespective of dose reductions due to AEs.

E1053
EFFECT OF PLASMA TROUGH CONCENTRATION OF NILOTINIB AND POLYMORPHISMS OF DRUG TRANSPORTER GENES ON THE FREQUENCY OF ADVERSE EVENTS IN CHRONIC PHASE OF CHRONIC MYELOID LEUKEMIA: STAT1 AND STAT2 TRIALS N. Takahashi1,*, T. Nioka2, M. Abumiya2, S. Takahashi1, M. Miura2
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Background: STAT trials (STAT1 and STAT2) are multicenter, phase II, single-treatment arm, open-label clinical studies designed to evaluate the efficacy and safety of two-year consolidation by nilotinib (NIL) for achieving a deep molecular response (DMR) or successful treatment-free remission (TFR) in patients with chronic phase chronic myeloid leukemia (CML).

Aims: In this report, we focus on the adverse events (AEs), especially anemia and liver dysfunction observed in the STAT trials. Additionally, we analyzed the relationship between laboratory abnormalities and pharmacokinetics (PK)/pharmacogenetics (PGx) of NIL.

Methods: AEs were assessed according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.03. Safety evaluations were conducted throughout the study. Plasma trough concentrations of NIL were determined with high-performance liquid chromatography (HPLC) at 1 month (1M), 3M, 6M, 12M, and 24M in the STAT trials. Genotyping of CYP3A5*3 [6986A>G (rs776746)], ABCB1 [3435T>C (rs1045642)], ABCG2 421C>A (rs2231142), and UGT1A1*6, *27, and *28 was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). All genotype frequencies were tested for Hardy-Weinberg equilibrium.

Figure 1.

Results: Between July 2011 and December 2012, CML patients were recruited in the STAT trials. NIL was administered twice daily (600mg/day) for 2 years according to the study protocol. A total of 76 and 96 patients were analyzed as a safety data set in STAT1 and STAT2, respectively. In STAT1, 18 patients who achieved a confirmed DMR were switched from STAT1 to STAT2. These patients entered both trials, but safety data had not been collected in STAT1 after entering STAT2 to avoid double counts. The PK/Pgx data of 147 of 154 patients were available and were evaluated in this study. Median trough concentrations of NIL were 1265 ng/ml at 1M, 1154 ng/ml at 3M, 974 ng/ml at 6M, 735 ng/ml at 12M, and 781 ng/ml at 24M. Although any-grade AEs were reported in patients in STAT1 and 55 patients in STAT2, the most common drug-related hematological and non-hematological AEs were elevated total bilirubin (28.6%), anemia (24.5%), elevated ALT (21.1%), and elevated AST (18.4%). The incidence of these AEs, except for anemia, was significantly associated
with high trough concentration of NIL (Figure 1). There were statistically significant correlations between median concentrations of NIL and the grades of each AE. Based on the results of the analysis using Cox proportional-hazards model, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), P=0.004] and ABCG2 421A/A [hazard ratio=3.044 (1.155-8.027), P=0.024] were independent factors for the elevated ALT. Similarly, the trough concentration of NIL [hazard ratio=1.001 (1.000-1.002), P=0.001] and UGT1A1 1′/1′ [hazard ratio=0.475 (0.246-0.919), P=0.02] were independent factors for the elevated total bilirubin.

Summary/Conclusions: In this study, we identified the relationship between NIL trough concentration and liver dysfunction. Our finding suggests that therapeutic drug monitoring might help avoid drug interruption and discontinuation because of AEs, especially liver dysfunction.

E1054

VERY EARLY MOLECULAR RESPONSE (VEMR) WITH FRONTLINE DASATINIB TREATMENT IS A STRONG PREDICTOR OF LONG-TERM BCR-ABL1 TRANSCRIPT LEVELS IN CHRONIC MYELOID LEUKEMIA PATIENTS: PCR-DEPTH STUDY

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Background: In BCR-ABL1 tyrosine kinase inhibitor (TKI) treated chronic phase chronic myeloid leukemia (CP-CML), early molecular response (EMR) at 3 months is currently identified as being one of the most important prognostic factors. Sokal risk score and dose intensity during first 3 months were strongly associated with achievement of EMR. As dasatinib is a novel kinase inhibitor with improved potency, identification of very early molecular response (VEMR) would be beneficial.

Aims: We evaluated the possibility of the VEMR at 1 month predicting long-term outcomes in newly diagnosed CP-CML patients treated with dasatinib.

Methods: In our prospective, observational, open-label study, 102 patients with CP-CML were enrolled to receive dasatinib at a dose of 100mg once daily. The primary end point was complete molecular response (CMR) by 18 months. Secondary end points including molecular response (MR) by 1, 3, 6, 12, 18, 24 month, time to and duration of MMR and CMR, and safety were tested. A receiver operating characteristic (ROC) curve from BCR-ABL1 transcript level on Day+28 was calculated to predict EMR and MMR at specific timepoints.

Results: Median age was 49 years (19-81 years) and 61 patients were male. With median follow-up duration of 28 months (0.9-33.8 months), 80 (78.4%) out of 102 patients were still on dasatinib treatment and 22 patients discontinued the dasatinib treatment. Tolerability (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%, respectively. In safety analyses, grade 1/2 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, fifty nine (62.1%) patients had less than 40% of BCR-ABL1 transcript (VEMR) on Day+28, thirty four (36.2%) patients achieved MMR at 12 months. However, only 27.8% (10 out of 36 patients) of patients without VEMR achieved MMR (p=0.001). 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, 48 (88.5%) patients achieved MMR at 24 months. However, only 48.2% (17 out of 35 patients) of patients without VEMR achieved MMR (p=0.001). Overall survival (OS) & progression-free survival (PFS) rates 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: A SYSTEMATIC REVIEW AND METAL ANALYSIS

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Background: PACE was the phase 2 single-arm trial of ponatinib, a 3rd-generation tyrosine kinase inhibitor (TKI), in 449 highly-refractory patients with CML Philadelphia-chromosome positive (Ph+) acute lymphocytic leukemia (ALL) or who had the BCR-ABL1 T315I mutation. Overall survival (OS) for 3L-CM 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-CM 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.

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<tr>
<th>Time (Years)</th>
<th>OS probability (95%CI)</th>
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<tr>
<td>1</td>
<td>90% (86%, 93%)</td>
</tr>
<tr>
<td>2</td>
<td>77% (72%, 83%)</td>
</tr>
<tr>
<td>3</td>
<td>66% (59%, 72%)</td>
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Summary/Conclusions: Estimated OS in patients with CP-CML receiving 3L treatment prior to ponatinib appears to be shorter than that observed among ponatinib-treated patients in PACE: 4-year survival probability in PACE was higher than estimated 2-year survival probability prior to ponatinib. Further analyses are needed to identify and adjust for potentially confounding factors.

E1056

DETECTION AND MONITORING OF BCR-ABL1 KINASE DOMAIN MUTATIONS IN CML AND ALL PATIENTS BY NEXT GENERATION SEQUENCING AND DROPLET DIGITAL PCR, A BELGIAN PROSPECTIVE STUDY

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Background: Among myeloproliferative diseases, development of chronic myeloid leukaemia (CML) is associated with the emergence of the fusion oncoprotein 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 treated with a Philadelphia chromosome (Ph+ ALL). Mutations of the BCR-ABL1 kinase domain constitute a major cause of treatment failure in CML and Ph+ALL patients receiving first or second generation tyrosine kinase inhibitors (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.

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.
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 worsening to all lines of TKI therapy according to the 2013 ELN-guidelines, with no suspected lack of adherence and ii) patients who had established MR4.0 diagnosis and/or molecular remission. Monitoring was performed when clinically appropriate.

Methods: Total BCR-ABL1 RNA was transcribed into a long range cDNA covering the kinase and the regulatory and the SH2/SH3 domains of either p190 or p210 BCR-ABL1 transcripts (exons 4 to 10). For NGS, primers designed with the Ampliseq™ Designer Software were generated as a set of 10 amplicons, bar-coded libraries, constructed according to the Ampliseq™ protocol, were sequenced on the Ion Torrent PGM platform (sensitivity of 2.5%). For ddPCR, cDNA was analysed for the presence of one of the 3 main mutations (T315I, E255K and Y253H). The overall number of BCR-ABL1 mutated samples was 18 (15 CML and 3 Ph+ ALL), representing 30% of the cases. Among these samples, 27 mutations were recorded. 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, reproductibility to determine mutational burden was found to be very high between NGS and ddPCR.

Summary: Advancements in sequencing technologies and further lowering sensitivity levels contribute to optimal management of CML and Ph+ ALL patients and improve treatment outcome. The earlier a mutation in the kinase domain is detected, the earlier an informed choice can be made regarding optimal subsequent TKI treatment.

E1057 CLINICAL AND IMMUNOLOGICAL EFFECTS OF NILOTINIB IN COMBINATION WITH PEGYLATED INTERFERON-Α2B IN PATIENTS WITH SUBOPTIMAL MOLECULAR RESPONSE ON IMATINIB (NORDUTCHCML009)

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Background: 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 have been approved, increasing the choice of TKIs for CP-CML patients, and lower disease progression rate than imatinib. On the other hand, longer treatment duration and the increased types of TKIs gave rise to various kinds of unexpected adverse events (AEs). In 2011, drug-induced peripheral arterial occlusive disease (PAOD) was first reported, followed by vascular AEs (VAEs) including peripheral arterial occlusive disease (PAOD) and central arterial occlusive disease (CAOD). Furthermore, it became clear that the incidence of VAEs increased with the dose and treatment duration, therefore VAEs are considered a more fatal complication of TKI treatment. However, there is no available data about the incidence of VAEs in Japanese patients.

Aims: We investigated the vascular safety issue and estimated the 1000 person-years risk of developing VAEs during TKI treatment, including imatinib, nilotinib, and dasatinib, using 3 risk assessment tools among 320 Japanese patients who were enrolled in the CML Cooperative Study Group.

Methods: A surveillance data of 320 patients enrolled in the CML Cooperative Study Group was conducted. This analysis was performed in Japanese patients. Among patients who were diagnosed with CML-CP from April 2001 to January 2016, whose median age was 57 years (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, Sita-score) to estimate the patients’ 10-year risk of VAEs.

Results: Among the 320 newly diagnosed CML-CP patients, 16 (5.0%) cases of VAEs were reported during the study period. Seven cases were treated by imatinib, 6 by nilotinib, 1 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 INH, 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 Sita-score (13 low, 1 intermediate, 5 high risk). The incidence rate of INH, CI, and PAOD was 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-
matched general population, respectively. Among the 320 newly diagnosed CML-CP patients, 16 (6.0%) cases of VAEs were reported during the study periods. Seven cases were treated by imatinib, 3 cases by nilotinib, 1 case by dasatinib, 4 cases were a switch from imatinib to nilotinib, and 1 case was a switch from dasatinib to nilotinib. The VAEs were 9 IHD, 5 CI, and 2 PAOD cases. Results from the 3 risk assessment tools are as follows: SCORE (2 low, 9 moderate, 1 high, 4 very high risk). Framingham score (3 low, 5 moderate, 7 high risk), and Suiita-score (13 low, 1 intermediate, 1 high risk). The incidence rate of IHD and CI per 1000 person-years were 5.26 and 2.92 in the enrolled CML-CP patients, 4.65 and 2.33 in the imatinib-treated patients, 14.34 and 10.75 in the nilotinib-treated patients, 4.08 and no case in the dasatinib-treated patients, and 1.787 and 3.342 in the age-matched general population, respectively.

Table 1.

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<thead>
<tr>
<th>Events</th>
<th>Frequency</th>
<th>Rate per 1000 years</th>
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<tr>
<td>IHD</td>
<td>19</td>
<td>5.26</td>
</tr>
<tr>
<td>CI</td>
<td>14</td>
<td>2.92</td>
</tr>
<tr>
<td>PAOD</td>
<td>5</td>
<td>0.14</td>
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Summary/Conclusions: The incidence rate of IHD per 1000 person-years were higher in the nilotinib- and lower in imatinib- and dasatinib-treated CML patients, and the patients showed almost the same rate of CI as compared with the age-matched general population, even though the incidence of VAEs were lower in Japanese compared to the European cohort. More patients were estimated to have very-high and high risk of VAEs in the SCORE and Framingham risk score assessment tools as compared with the Suiita-score tool.


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Background: Since the introduction of Tyrosine Kinase Inhibitors (TKI), many patients diagnosed of Chronic Myeloid Leukemia (CML) in chronic phase achieve a deep molecular response. Around 50% of these patients are expected to maintain their molecular responses even after discontinuation of their TKI treatment. Several clinical trials are exploring the best way of stopping TKI therapy and evaluating patient and disease characteristics that could predict relapse after treatment discontinuation.

Aims: This is an update of the CMRegistry study aimed at collecting clinical data and molecular information from Spanish CML patients that have achieved a series of molecular milestones to any of the tyrosine kinase inhibitors in order to monitor their progress and the achievement of a stable deep molecular response: >MR4 (IS).

Methods: CMRegistry is an observational, multi-center and prospective study. CML patients treated with 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 bcr/abl ratio of: 1) ≤1% at 6 months from start of TKI therapy, or 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: 621 patients low risk, 307 intermediate risk and 129 high risk. Cutoffs 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 SAFE-TY BASED ON BASELINE COMORBIDITY AND AGE IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP) IN DASISION G. Saglio17, J.E. Cortes2, A. Hochhaus3, N.P. Shah4, E.L. Atallah5, M. Abakharouf6, L. LP, M.J. Mauro7

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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 comorbidities (Khoury ASH 2010). Further analysis is warranted to determine how comorbidities may impact long-term outcomes.

Aims: To evaluate the impact of baseline comorbidities and patient age on 5-year safety and efficacy in dasatinib- or imatinib-treated patients from DASISION.

Methods: In DASISION (NCT00481247), patients were randomized to receive dasatinib 100mg/day (N=259) or imatinib 400mg/day (N=260). For this retrospective analysis, patients were grouped as having 0 or ≥1 baseline comorbidity, by baseline disorder (diabetes mellitus, hepatobiliary disease, hyperlipidemia, cardiovascular disorder, or pulmonary condition), or by age group (<46 years, 46–65 years, >65 years). Safety (treatment-related AEs in ≥10% of patients) and efficacy (response rates by 5 years and median times to response) were assessed for each group and treatment.

Table 1.
Results: The number of patients with 0 or ≥1 comorbidity was similar in the dasatinib (66 [25%]; 193 [75%]) and imatinib (67 [26%]; 193 [74%]) arms, respectively; most (>90%) patients were <65 years old. In patients with 0 or ≥1 baseline comorbidity, the median average daily dose was comparable within arms and discontinuation rates (36%-39%) were similar within and across arms (table). The overall safety profiles were comparable in the 0 and ≥1 comorbidity groups in both arms, other than specific AEs, which had a 22 times higher frequency in patients with ≥1 vs 0 comorbidities; the majority of these were grade 1/2 AEs (table). The incidence of peripheral edema increased with patient age for both imatinib and dasatinib (<46 years: 5% each; 46–65 years: 12% and 10%; ≥65 years: 21% and 20%). In this analysis, the increased incidence of pleural effusion (PE) in the 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 for patients treated once daily, but were numerically 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 (36 or 35) vs imatinib (MR4.5: 42 or 47).

Summary/Conclusions: The superior efficacy of dasatinib over imatinib was shown in previous studies. Response rates and times to response were comparable in patients with 0 or ≥1 comorbidity and trended in favor of dasatinib vs imatinib. Although a few AEs (most grade 1/2) appeared to occur at a higher frequency in patients with ≥1 vs 0 comorbidities in either treatment arm, the overall occurrence 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 LEUKAEMIA PATIENTS WITH RESISTANCE OR INTOLERANCE TO BCR-ABL TKIS: 36 MONTHS UPDATE OF RADOTINIB PHASE 2 STUDY

Background: Radotinib is an orally active, selective BCR-ABL1 tyrosine kinase inhibitor (TKI), approved for the first-line and second-line treatment of chronic phase chronic myeloid leukemia (CP-CML) patients in Korea. Earlier 12 and 24 month results demonstrated that radotinib is effective and well tolerated in CP-CML patients with resistance and/or intolerance to BCR-ABL TKIs.

Aims: We update the long-term outcome of radotinib treatment in patients failed to 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 base-line, every 3 months, and at treatment failure. Safety parameters were also analyzed. Probabilities of overall survival (OS) and progression free survival (PFS) were calculated using Kaplan-Meier method.

Results: A total of 77 CP-CML patients (18 years of age or over) were enrolled. This analysis includes data from last enrolled patient who received at least 36 months of radotinib therapy. With a median follow-up of 45.7 (range 0.9-65.7) months, 31 patients (40.3%) completed 36 months treatment, and 46 patients (59.7%) discontinued the treatment before 36 months. Main reasons of discontinuation were abnormal laboratory test (n=18), adverse events (n=4), treatment failure including disease progression and lack of response (n=18), death (n=2), and other reasons (n=4). Median duration of radotinib exposure was 19.5 (0-36.9) 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 1,7

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 through. 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 through. 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 Econometrics 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 Econometrics 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 2010 and 30 in 2030 where the tendency inflects, and 30 after 2040. Considering the 100% relative survival hypothesis, a target CML prevalence were 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 inhabitant.

E1064

THE ROLE OF MICRORNAS 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 studied the research of the mechanisms involved. These included those related with target changing (e.g. the presence BCR-ABL gene mutations and amplifications) and with intracellular drug concentrations (e.g. the normal levels of influx and efflux transporters such as OCT1/OCTN2 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-RD). K562-RC cells, generated by continuous exposure to Imatinib, presented an 8 times higher BCR-ABL levels (K562; 100% of 26, K562-RD cells (created by discontinuous exposure), the degree of resistance is 18x. Statistical analysis was performed with ANOVA and multiple comparison tests, with significance levels of 95% (p<0.05).

Results: The miR-203 and miR-518c expression was not detected in any cell line and in patient samples. First, we examined miRs expression with BCR-ABL levels. Higher levels of tumor suppressor miR-451 were associated with a higher reduction of BCR-ABL levels (lower than 0.01%) in CML patients and patients with higher BCR-ABL present lower levels of expression of miR-451. This miR was also down-regulated in LAMA-84, K562-RC and K562-RD comparing with sensitive cell line (K562; 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, mi21 and miR-26. These miRs were also up-regulated in resistant cell lines. MiR-21 was more relevant for K562-RC cells (4-fold higher than K562). LAMA-84 and K562-RD cell lines showed almost 2 times more expression of miR-21. 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 as 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 predicts 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.
(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 (1297T>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, treatment discontinuation for primary or secondary resistance or intoler ance. PFS was calculated from the start of TKI to ABP or death.

Results: A total of 196 patients with CP-CML (median age 57 years, range 21-84) were included in the analysis. Frequency of ABCB1 and ABCG2 SNPs expression is summarized in Table 1. Considering response to therapy, either in imatinib-treated patients and in those receiving a 2G-TKI, we did not find any significant difference in terms of optimal response at the various timepoints, TTF or PFS for ABCB1 C1236T, G2677T and C3435T and of ABCG2 G34A and C412A polymorphism, even if there was a trend for a worse PFS in the few patients (n=2) with 1236 allele A treated with imatinib. Conversely, we found a lower rate of optimal response at 3 (p=0.1), 6 (P=0.05) and 12 (p=0.02) months in imatinib-treated patients with TC genotype of ABCB1 T129 SNP, though the small number of patients (7) had probably and impact on statistical significance. However, TTF was shorter for ABCB1 1297C allele C patients, both receiving imatinib (P=0.05) and 2G-TKIs (P=0.07), and also PFS was significantly shorter in this cohort (P=0.003).

Table 1.

<table>
<thead>
<tr>
<th>MDR protein</th>
<th>SNP</th>
<th>Genotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCB1</td>
<td>C1236T</td>
<td>CT</td>
<td>32%</td>
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<tr>
<td></td>
<td>G2677T</td>
<td>GG</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>C3435T</td>
<td>GC</td>
<td>28%</td>
</tr>
<tr>
<td></td>
<td>T129</td>
<td>TC</td>
<td>32%</td>
</tr>
<tr>
<td>ABCG2</td>
<td>G34A</td>
<td>GA</td>
<td>32%</td>
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<tr>
<td></td>
<td>G21A</td>
<td>GA</td>
<td>33%</td>
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</tbody>
</table>

Summary/Conclusions: With the limits of the low expression rates of some SNPs, our data suggest a lower response in patients harboring 1297C 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 ASSESSMENT TO EUROPEAN TREATMENT AND OUTCOME STUDY (EUTOS) SCORE


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Background: The discovery of tyrosine kinase inhibitor (TKI) imatinib has revolutionized the conception of chronic myeloid leukemia (CML) as a mortal disease to a long-term controllable disease. The European Treatment and Outcome Study (EUTOS) score is a clinical tool that utilizes imatinib-based objectives to predict treatment response and progression free survival (PFS) in patients with CML in chronic phase (CP). However, it is currently unknown whether the introduction of second generation TKIs (2nd TKIs) affects prognostic score of patients with CML-CP, particularly among those considered high-risk according to EUTOS score.

Aims: Our study aims to highlight the critical role of the introduction of 2nd TKIs on patient prognosis as determined by EUTOS score.

Methods: Patients data was obtained retrospectively from patients enrolled in the CML Cooperative Study Group. Patients with CML-CP who were treated with any TKIs as first line therapy between April 2001 and January 2016 were enrolled to the study and were classified according to date of diagnosis. Those who were diagnosed with CML-CP before March 2009 were classified into the imatinib group, and those diagnosed after April 2009 were classified into the 2nd TKI group. The study was approved by the research ethics boards of each institution and was conducted in accordance with the Declaration of Helsinki.

Results: There were 308 patients newly diagnosed with CML-CP during the study period. Of these patients, 104 (34%) were assigned to the imatinib group and 204 (66%) were assigned to the 2nd TKI group. With respect to EUTOS score, 223 patients were classified as low-risk, of which 69 were in the imatinib group and 154 were in the 2nd TKI group. Forty-six patients were considered high-risk, of which 19 were in the imatinib group and 27 were in the 2nd TKI group. EUTOS score was unavailable in 39 patients. With regard to initial TKI, all patients were treated with imatinib in the imatinib group. Among patients assigned to the 2nd TKI group, 149 (73%) were initially treated with any TKI. The median follow-up period for all patients was 48 months (range: 1–185 months). Among patients in the 2nd TKI group, CML-associated death rates were significantly lower than those in the imatinib group. EUTOS high-risk patients score exhibited significantly worse outcomes in EFS, PFS, and CML-associated death compared to those considered low-risk. Most importantly, risk stratification by EUTOS score was predictive of risk-associated clinical outcomes in patients assigned to the imatinib group; however, EUTOS score failed to predict risk-associated clinical outcomes of patients assigned to the 2nd TKI group (see Figure). The EUTOS high-risk patients in the imatinib group showed worse clinical outcomes than those in the 2nd TKI group (hazard ratio [HR] 6.35, 95% confidence interval [CI] 1.79 – 22.6, p=0.0042). However, prognostic effect was less in the 2nd TKI group (HR 3.21, 95% CI 0.59 – 17.6, p=0.18).

Conclusions: Among patients assigned to the imatinib group, risk stratification by EUTOS score was predictive of clinical outcomes in that those considered high-risk experienced considerably more adverse events (EFS, PFS, or CML-associated death) than those considered low-risk. Our results support the use of 2nd TKIs in treating high-risk patients with CML-CP in order to avoid disease progression. Future large-scale studies are necessary to evaluate the clinical significance of EUTOS scoring in the accurate prediction of prognosis among patients with CML-CP treated with 2nd TKIs.

Figure 1.
CHRONIC MYELOID LEUKEMIA DIAGNOSED DURING PREGNANCY: THERAPY TACTICS AND OUTCOMES
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Background: Chronic myeloid leukemia (CML) diagnosed at pregnancy is a serious challenge. Treatment by tyrosine kinase inhibitors (TKI) today is considered harmful for fetus due to possible teratogenicity. On the other hand TKI delay is dangerous for disease progression as no other options have comparable to TKI effectiveness. Pregnancy termination by abortion may be crucial for desired pregnancies as further childbirth is postponed for years until stable deep molecular response (DMR). Due to limited number of cases and ethical issues there is no consensus on how to behave in such delicate cases.

Aims: To describe pregnancy outcomes and therapy tactics for CML diagnosed at pregnancy.

Methods: Information regarding CML diagnosed at pregnancy was collected with the participation of 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 1.

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 respectively, no data for risk score was in 1 patient. CML was diagnosed with Ph-positive chronic phase CML during pregnancy. That was 11% of other KIR3DL1 alleles. (Fisher’s exact test, p<0.001).

E1068 IMPACT OF KIR3DL1*00501 IN TYROSINE KINASE INHIBITOR-TREATED CML
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Background: The BCR-ABL1 tyrosine kinase inhibitors (TKIs) dramatically improved long-term survival of the patients with chronic myeloid leukemia (CML). As increased NK cells during TKI therapy positively correlate with better outcome, antitumor immunity by NK cells may contribute to the effects of TKIs. However, the response to TKIs depends on each case, and the determinants of it remain to be elucidated.

Aims: Given that NK cell function is regulated depending on the interaction between killer immunoglobulin-like receptor (KIR) and human leukocyte antigen (HLA) class I molecules, we hypothesized that polymorphisms of KIR and HLA play important roles in the responses to TKIs. Then we performed allele genotyping of KIR and HLA with deep sequencing in CML patients, and here report our findings.

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 µg RNA in TMA method. We also defined DMR as 4-log reduction (MR4) in RQ-PCR (IS), which is similar to undetectable of BCR-ABL transcript level in TMA method.

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 therapy, 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 of therapy; KIR2DL4*008 or 011/00501, and 2DS4*00301 or 007/010 or 015 than 2DL4*007/008 or 009/010 (HR 1.973, 95% CI, 1.310 to 3.000, p=0.001); KIR2DS4*00301 or 007/010 or 015 (HR 2.811, 95% CI, 1.658 to 4.968, 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 of therapy; KIR2DL4*008 or 011/00501, and 2DS4*00301 or 007/010 or 015 than other KIR3DL1 alleles. (Fisher’s exact test, p<0.001).

Summary/Conclusions: KIR3DL1*00501 and several KIR2DL4 and 2DS4 alleles positively correlate with better therapeutic effects of TKIs, and they may form the same KIR haplotype. Our data indicate that these KIR alleles represent strong anti-CML immunity by NK cells, and consequently may associate with long-term outcome and treatment-free remission in CML.

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 become 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. Aims: To evaluate the second IM discontinuation (Korean imatinib Discontinuation Study; KID Study), we have explored molecular kinetics after the first IM discontinuation and after IM resumption for molecular relapse. In patients regaining durable UMRD with IM resumption, we tried second IM discontinuation and compared molecular kinetics between the first IM stop and second IM stop.

Methods: CP CML patients who were treated with IM for more than 3 years and had undetectable levels of BCR-ABL1 transcripts determined by quantitative reverse transcriptase-polymerase chain reaction (PCR) for at least 2 years were eligible for Kid Study and in cases of MMR loss after 2 consecutive assessments, IM treatment was re-introduced. After IM resumption for MMR loss, IM treatment was re-introduced. The patients who lost UMRD until IM resumption were included with a median time of 45 years (range, 25-59 years) entered into a second IM discontinuation after maintaining UMRD at least 2 years. Prior to first discontinuation, the median duration of IM therapy was 68.9 months (range, 38.5-115.1 months) and the duration of sustained UMRD was 32.9 months (range, 24.8-64.5 months. After first attempt of IM discontinuation, they relapsed after a median duration of 3.7 months (range, 1.5-11 months) and second IM discontinuation was attempted after maintaining UMRD for 6.7 months (range, 3.3-13.6 months) after IM resumption. After sustaining a second UMRD for a median of 25.5 months, IM therapy discontinued for a second time. After a median follow-up of 8.8 months (range, 0.3-38.1 months) after second IM discontinuation, 10/12 patients (83%) and 8/12 patients (67%) lost UMRD and MMR, respectively. Among two consecutive studies (korean imatinib Discontinuation Study; KID Study), MMR loss was similar to those of the first discontinuation. But the molecular kinetics after MMR loss, except one patient, were retreated with IM for 9.4 months and another patient have shown gradually increasing BCR-ABL1 transcripts under the level of 0.1% on IS. 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: Though the Korean multicenter prospective study (Korean Imatinib Discontinuation Study) exploring second imatinib discontinuation is limited, our data demonstrated that a second attempt might be possible and the median time to MMR loss after second discontinuation was similar to those of the first discontinuation. But the molecular kinetics after second ISM loss needs longer follow-up with more patients. Further studies on the predictors to select patients for a trial of second TFR and novel strategies such as intermittent therapy will be warranted.

E1071

HYDROXYUREA SUPPRESSES BCR-ABL1 T315I+ CML CLONES IN VIVO AND IN VITRO AND SYNERGIRES WITH PONATINIB IN ELIMINATING TKI-RESISTANT CML CELLS

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Background: In chronic myeloid leukemia (CML), BCR-ABL1 T315I leads to resistance against most BCR-ABL1 tyrosine kinase inhibitors (TKIs). Long-term therapy with ponatinib, which suppresses BCR-ABL1 T315I, is problematic due to occurrence of compound mutations in BCR-ABL1. Therefore, alternative therapies have to be considered. Hydroxyurea (HU) has been used for (palliative) treatment of CML over decades. However, the effects of HU on TKI-resistant sub-clones have not been examined so far.

Aims: The aim of this study was to evaluate the effects of HU on CML subclones carrying BCR-ABL1 T315I mutations (isolated or in compound-configuration) in vitro and in vivo and to explore cooperative effects between HU and ponatinib.

Methods: Four BCR-ABL1 T315I+ CML patients were treated with HU (1-3 g/day) for 2 to 18 months. White blood counts (WBC), differential counts, and BCR-ABL1 transcript levels were reported. The BCR-ABL1 T315I+ BCR-ABL1 ratio was determined by mutation-specific, ligation-dependent, PCR and next generation sequencing. In vitro, cells were cultured. In vivo, patients were randomized 1:1:1 to radotinib 300mg bid (n=79), radotinib 400mg bid (n=81), or imatinib 400mg once daily (qd) (n=81). 157 patients with available 3 months qRT-PCR on study therapy (radotinib 300mg bid (n=79), and imatinib 400mg qd (n=78)) were evaluated. And, total of 151 patients who received radotinib 300mg bid group were significanlly higher MMR and MR 4.5 compared with the patients who were not MMR and MR 4.5 by 24 months: 73.5% and 38.2% in the radotinib 300mg bid group and 63.6% and 29.1% in the imatinib group, respectively. At 6 months, 73.4% of patients in the radotinib 300mg and 53.1% patients in imatinib group (P=0.0246) achieved 6 months EMR. The patients who had EMR at 6 months in radotinib 300mg bid group were significantly higher EMR, longer follow-up are needed.

Summary/Conclusions: With minimum 24 months follow-up, early responses at 3 months or 6 months can predict better outcomes in newly diagnosed chronic myeloid leukemia patients treated with radotinib or imatinib. But, to evaluate the significant long-term prognostic value such as overall survival and progression-free survival by EMR, longer follow-up are needed.
HU+ponatinib for 72 hours, and the percentage of viable cells in each subclone was analyzed by flow cytometry.

**Results:** HU treatment resulted in WBC stabilization in 3 of 4 patients, but failed to induce a molecular response. However, surprisingly, the percentage of *BCR-ABL* diminished significantly in all 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* 1315. In 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-ABL* were more sensitive against HU than cell lines expressing *BCR-ABL* WT. HU and ponatinib were found to synergize in inhibiting growth of all cell lines tested, including cell lines expressing *BCR-ABL* 1315 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/F3p210*WT* cells but not Ba/F3p210*1315F* or Ba/F3p210*T315I* cells whereas HU was found to exert stronger effects on cells expressing mutant *BCR-ABL* 1. The drug combination resulted in complete suppression of all sub-clones.

**Summary/Conclusions:** Our data show that HU exerts strong, sub-clone specific, additive effects on TKI-resistant cells. HU+ponatinib produce synergistic growth-inhibitory effects on TKI resistant CML cells. Clinical studies are now warranted to define the exact value of the drug combination ponatinib+HU in TKI resistant CML.

**E1072**

**ASSOCIATION OF BCL2L11 (BIM) DELETION POLYMORPHISM WITH MOLECULAR RELAPSE AFTER TYROSINE KINASE INHIBITOR CESSION IN CHRONIC MYELOID LEUKAEMIA 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. However, no predictive prognostic factor for successful treatment cessation has yet been identified. BCL2L11 (BIM) deletion polymorphism (intron 2) has been reported to be associated with an inferior response to TKI (Ng et al. Nature Medicine, 2012). We have previously reported that CML cell lines with BIM deletion polymorphism may predict relapse after TKI discontinuation (Katagiri et al. Br J Haematol, 2013).

**Aims:** To further clarify the role of predictive biomarkers in molecular relapse after TKI cessation, we performed a long-term follow-up of CML patients with DMR after TKI cessation.

**Methods:** With patients receiving TKI treatment were included. Molecular relapse was defined as a loss of the major molecular response (MMR). The genomic DNA of patients was obtained from their whole blood samples using the EZ1 DNA Blood 350 kit (Qiagen, Valencia, USA). Deletion polymorphism was detected by Q-Invader assay using primers designed to detect a deletion site (5’-GCTTATGGATGCGCTCTT-3’) (Ohyashiki et al. J Hematol Transfus, 2014).

**Results:** Forty-six CML patients (29 men;17 women; median age, 58.5 years) were included in this study (Sokal category; low: 32; intermediate: 10; and high: 2). Thirty-three patients discontinued imatinib, five discontinued nilotinib, and eight discontinued dasatinib. Ten patients were treated with IFNα before TKI treatment. The median duration from TKI initiation to cessation was 85.0 (range: 22–177) months; the median duration of DMR before TKI cessation was 43.0 (range: 5–131) months. Treatment-free remission was estimated to be 66.5% at 12 months, 61.5% at 24 months, and 58.5% at 36 months. Thirty-six CML patients were analyzed for the presence of BIM deletion polymorphism in six cell lines or cell-line derived RNA studies for both the b3a2 and 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 32,000 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.

**E1073**

**XPERT® BCR-ABL ULTRA, A HIGH SENSITIVITY ASSAY WITH A LIMIT OF DETECTION REACHING MR4.5 AND BELOW ON AN INTERNATIONAL REPORTING SCALE**

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**Background:** The ability to consistently detect low levels of *BCR-ABL* transcripts in patients with chronic myeloid leukemia (CML) is important in the assessment of treatment outcomes in patients on tyrosine kinase inhibitor (TKI) therapy. Particularly, *BCR-ABL* assays that are sensitive in the measurement of deep level response may aid in the identification of potential candidates for treatment discontinuation. Xpert® BCR-ABL Ultra detects the most common *BCR-ABL* transcripts below MR4.5 (Molecular Response at 4.5-log reduction) or 0.0032%, which is widely accepted as the clinical threshold that defines candidates who can safely discontinue TKI therapy.

**Aims:** The present studies were designed to verify the limit of detection (LoD) for Xpert® BCR-ABL Ultra assay below MR4.5 on the International Scale (IS) in clinical samples for both the b3a2 and b2a2 transcripts.

**Methods:** To overcome the challenge of testing numerous replicates requiring large volumes of patient samples, serial dilutions ranging from BCR-ABL/ABL levels of 10% to <0.001% (IS) were prepared as contrived samples using CML samples with initial BCR-ABL level >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 32,000 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.

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E1074
IDENTIFICATION OF INCIDENTS CASES OF GAUCHER DISEASE IN SPLENOMEGALY AND/OR THROMBOCYTOPENIA PATIENTS IN SPECIALIZED MEDICAL SERVICES IN COLOMBIA THROUGH THE USE OF A SELECTION ALGORITHM

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 <11 g/dl in men and <11 g/dl in women) plus/or bone pain plus/or Monoclonal Gammapathy of Unknown Significance plus/or Polyclonal Gammapathy in subjects aged 30 years and older; and/or splenomegaly defined as palpable spleen ≥1 cm 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, or with other congenital 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, confirmation with bone marrow aspirate was performed. Both enzymatic activity in leucocytes was determined. GBA1 gene was analyzed.

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% women) 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/or thrombocytopenia referred to Hematology, Pediatric Hematology, Pediatrics and Internal Medicine, using a selection algorithm for the generation of diagnostic algorithms to support medical specialties in Latin America 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.

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.

Acknowledgements: This study was funded by Sanofi Genzyme Colombia and coordinated by Caimed Colombia.

E1075
IMPACT OF PEROXIREDOXIN 2, GLUTATHIONE PEROXIDASE AND CATALASE INHIBITION ON OXIDATIVE STRESS MODIFICATIONS OF RED BLOOD CELL MEMBRANE AND CYTOSOL

Background: In oxidative stress conditions, the interplay between catalase (CAT), glutathione peroxidase (GPx), and glutathione antioxidant enzymes is key for the maintenance of the red blood cell (RBC) oxidative stress defense. Peroxiredoxin2 (Prx2) has been shown to act as a specific Prx2 inhibitor. The aim of this study was to assess the ability of Prx2 in the inhibition of CAT, GPx, or a mixture of these enzymes in with or without secondary peroxidase inhibition.

Methods: We performed in vitro assays (n=3) with RBCs from healthy volunteers, inhibiting Prx2, GPx and CAT, either individually, two-by-two or all three; conodin A, mercaptosuccinic acid and sodium azide were used as specific inhibitors of Prx2, GPx and CAT, respectively. Since the RBC membrane is a major target of ROS, we evaluated membrane lipoperoxidation (LPO) and membrane bound haemoglobin (MBH), as well as, cytosol’s total antioxidant status (TAS), by spectrophotometric methods.

Results: Concerning TAS we found a trend towards decreasing values with enzyme inhibition (one or more); the lowest value of TAS was observed when all three enzymes were inhibited and, when only two enzymes were inhibited, the lower values were obtained for pairs that included CAT inhibition; when only one enzyme was inhibited, GPx inhibition showed the lowest LPO and membrane bound haemoglobin (MBH), as well as, cytosol’s total antioxidant status (TAS), by spectrophotometric methods.

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...
MOLeULAR 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_002986.5) including exon-intron boundaries were selectively amplified and followed by direct Sanger sequencing.

Table 1.

Results: Seven index PK def. patients as confirmed by enzyme activities, age range 9-35 yrs old, were identified (Table 1). Three patients presented with severe hemolytic anemia and required regular blood transfusion; every 3-4 weeks in two (PK-1 and PK-3) and every 10-12 weeks (PK-2) in which one patient (PK-1) has been successfully treated with bone marrow transplantation and became transfusion-free. Three patients (PK-5, 6 and 8) had moderately severe hemolytic anemia and required blood transfusion occasionally. Only one patient (PK-7) from Indonesia had well-compensated anemia and never required blood transfusion. All but one had PK activities lower than 50% of normal range but these activities did not correlate with clinical severity. We found 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.865C>T=p.R289W; c.1618G>T=p.G540X). One mutation (c.941T>C=p.I314T) seemed to be recurrent since it was found in two families; one homozygous and one compound with N118S. Beside nucleotide mutations, we found a 5006 bp deletion from intron 3 to exon 10 affecting PKLR gene. To detect these mutations in family members and further cases, we developed a long range GAP-PCR analysis to amplify the breakpoint fragment and directly sequenced to determine deletion extends and also ARMS-PCR (C.1641T>TA, PCR-RFLP (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.

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).

Aims: Since pediatric patients with splenomegaly and cytopenias are usually referred to pediatric hematologists, we have designed the GAU-PED study to consider GBA activity in cellular homogenates. A pediatric algorithm has been proposed to promote timely diagnosis and early access to ERT (figure 1).

Figure 1.

Aims: Since pediatric patients with splenomegaly and cytopenias are usually referred to pediatric hematologists, we have designed the GAU-PED study to
evaluate the prevalence of GD among children referred to the haematology paediatric units and selected according to the above mentioned diagnostic algorithm. Here, we report a preliminary analysis of GAU-PED trial.

Methods: The GAU-PED study involves 53 centers in the context of the AIEOP Study Group, the Italian clinical research consortium in paediatric haematology and oncology. Patients referring to the pediatric hematology and oncology units for a referral diagnosis of haemolytic anaemia (thrombocytopaenia, haemolytic anaemia and/or anaemia), where other causes of splenomegaly have been excluded, are tested for GBA activity though a DBS sample. Only patients with DBS showing a GBA activity below normal values are recalled to confirm GBA enzyme deficiency using the gold standard GBA analysis in cell omogenate. For every tested patient, detailed 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/h-1 were found in 9/25 patients (36%). These patients have been recalled for the conventional enzymatic test. The diagnosis of GD has been confirmed in 5/9 patients (55%), with a prevalence of GD of 20% (95% CI: 8.6-39.1%) equal to 5/25 patients in the tested population. In all 5 patients the genetic analysis has been consistent with GD. Three patients were males and 2 females. The mean age at diagnosis was 8 years (range 2-13 years). The median time from the initial clinical presentation and diagnosis has been 12 months (range 6-50 months), while the mean time between the DDS 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 thrombocytopaenia considered at increased risk for the disease. The use of a GAU-PED diagnostic algorithm is useful to increase awareness of GD among pediatric hematologists and to shorten the time to diagnosis. Taking into consideration the long life expectancy of pediatric GD patients, the early diagnosis will have a strong impact on health and quality of life.

E1079

CIRCULATING MICROPARTICLES IN CONGENITAL AND ACQUIRED HAEMOLYTIC ANAEMIA

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Background: MPs play an important role in several biological processes. Elevated levels of circulating MPs have been described in several haemolytic diseases and other clinical conditions. MPs are tested for GBA activity though a DBS sample. Only patients with DBS show positive结果s.

Methods: To evaluate platelet MPs (PMP), tissue factor expressing MPs (TFMPs), endothelial MPs (EMPs) and microparticles expressing single antigens (CD41, CD144) levels in other haemolytic anemias, as HERV-s pherocytosis (HS), elliptocytosis (HE), stomatocytosis (HSt), red cell enzymatic defects, congenital dyserythropoietic anaemia (CDA), autoimmune haemolytic anaemia (AIHA), and paroxysmal nocturnal haemoglobinuria (PNH). The number of MPs here investigated were correlated with Hb levels (p<0.05), and PMPs positively correlated with Hb (p<0.05). Finally, the number of annexin V APC was increased in PKD compared with controls (p=0.023), and positively correlated with disease duration (r=0.999, p<0.001). PMPs and TF-MPs were elevated too, although not significantly. The number of MPs here investigated were comparable between splenectomized and not splenectomized, and between naive and treated patients.

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.
PIEZ01 MECHANOTRANSDUCTIVE PROTEIN MUTATIONS IN RBCS: WHEN THE PHENOTYPE IS BEYOND HYEMOLYTIC ANAEMIA

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Background: Piezo proteins are integral membrane proteins with many transmembrane domains broadly expressed, including erythrocytes (RBCs). PIEZ01 proteins play an important role as an osmoreceptor, maintaining RBCs ionic homeostasis, functioning as a mechanically activated cation channels. Mutated PIEZ01 proteins have been linked to hereditary xerocytosis (HX), which is characterized by RBCs dehydration with mild to moderate compensated haemolytic anaemia and iron overload. As these clinical features are present in many different clinical conditions, the diagnosis always needs a high level of suspicion. Nowadays, besides peripheral blood smear (PBS) observation, molecular analysis, searching for mutations in PIEZ01 gene, became a tool in the diagnosis of HX.

Aims: Describe 26 patients with HX associated with PIEZ01 mutations belonging to 13 unrelated families, raising awareness of the highly variable phenotype of this patients, and the need of a high grade of suspicion along with the morphologic evaluation of the PBS.

Methods: Collection of clinical and laboratory data on our 26 patients with HX and hyperferritinaemia due to 10 different identified mutations in PIEZ01. Sanger sequencing was used to identify mutations affecting PIEZ01, encoded by FAM38A gene, and to confirm transmission according to the presence of disease phenotype. In all were included other known causes of hyperferritinaemia (HF) and haemolytic anaemia.

Results: Of the patients identified having PIEZ01 mutations, 13 were probands and 13 were identified by family studies. Median age at diagnosis was 43 years (1-80), with female predominance (n=14; 53.9%). 4/13 probands had family history of HX (n=1) or HF (n=2). The common feature of our entire cohort of patients was the presence of xerocytoses in PBS. 13/26 patients had reticulocytes counts of one or less (normal <10%), 16/26 patients had Hb with a mean value of ferritin of 556ng/mL (161-617) and 9/26 had both. Of the 26 patients, four had splenomegaly and six gallbladder lysisis (5/6 cholecystectomized), two of them have both. Only 5 patients presented with anaemia (Hb <12g/dL), 2 macrocytic and 3 normocytic. One patient that presented with anaemia also has abnormal spherocytosis carrier. We are detecting 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 xerocytoses 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 xerocytoses 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 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 the presence of xerocytosis in PBS and increased haemoglobin affinity for oxygen and a high reticulocytosis. We studied the hemoglobin oxygen affinity parameters in HX patients.

Results: All the 14 HX patients carried one or two missense mutations in PIEZ01, no gene variation was identified in KCNN4 or FAM38A. 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 21, 14 of 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 PIEZ01 mutated HX. Of note, p50 was not correlated with the Hb level (mean 139, range 112-180 g/L in HX patients versus 125, range 93-142 g/L in HS patients). Intracellular red cell 2.3 DPG level could be measured in 7 HX patients from 4 families, it was found decreased in all of them (0.43+/-0.06, normal 0.9+/-0.19), providing a pathophysiological basis for the increased hemoglobin affinity we observed. In particular, we observed a significant increase in the hemoglobin affinity for oxygen restricted to PIEZ01 mutated HX.

Summary/Conclusions: The increased hemoglobin affinity for oxygen observed in HX patients reflects the decrease in the 2.3 DPG level. This may be a consequence of a high ATP requirement and an increased glycolytic activity in HX red cells at the expense of the 2.3DPG shunt in order to maintain the cell ion homeostasis. High hemoglobin affinity for oxygen may induce a relative tissue hypoxia and consequently a high reticulocytosis, providing a clue to explain the compensated hemolysis frequently observed. However, the links between PIEZ01 mutations, red cell enzymatic activity and erythropoiesis need to be clarified and a proteomic and a metabolomic approach is under investigation. Of interest is the clinical point of view, HX diagnosis is sometimes difficult and this low p50 value, easily measured on venous blood, represents a useful new diagnosis tool for HX.
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 I (BK), and fungal infections, mainly by Aspergillus Fumigatus (Asp), are leading causes of transplant-associated mortality in patients undergoing allogeneic hematopoietic stem cell transplantation. Standard treatment with antiviral and antifungal pharmacological agents, is often ineffective or toxic and may lead to resistance. Due to these limitations, adoptive immunotherapy with antigen-specific T cells has emerged as an attractive alternative. Towards unleashing its full potential and treat multiple viral and fungal infections by a single T-cell product, we developed a rapid, simplified and minimally laborious protocol for the generation of multipathogen-specific T cells (mp-STs) that simultaneously target CMV, EBV, BK and Asp, from healthy donors.

Aims: Due to the lack of mouse models recapitulating the clinical condition of multiple opportunistic infections in transplanted hosts, we here aimed to test the in vivo safety of produced mp-STs and provide a proof of concept of their efficacy in a humanized model of invasive aspergillosis (IA).

Methods: mp-STs were generated from healthy donors by pulsing 1.5x10^7 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 immunomagnetically isolated CD3+cells (donor lymphocyte infusions-DLI) or mp-STs were infused in myelo/immuno-ablated NSG mice which had been intra-nasally inoculated with Asp conidia or left uninfected. Cells were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assessed by histology and immunohistochemistry.

Results: We generated 23x10^7 cells mp-STs (12-fold expansion). All cell lines were polyclonal expressing constitutive receptor memory markers and specific against Asp (spot forming cells [SFC]/2x10^5 cells: 315±82) and the targeted viruses, if derived from seropositive donors [SFC/2x10^5 cells, CMV: 637±267; EBV: EBNA1, LMP2, BZLF1; BK: Large T, VP1) and Asp pepmixes (Crf1, Ge1, SHMT) and culturing for 10 days. The specificity of mp-STs was analyzed by IFN-γ Elispot. A total of 1.5x10^7 immunomagnetically isolated CD3+cells (donor lymphocyte infusions-DLI) or mp-STs were infused in myelo/immuno-ablated NSG mice which had been intra-nasally inoculated with Asp conidia or left uninfected. Cells were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assessed by histology and immunohistochemistry.

Summary/Conclusions: Overall, engrafted mp-STs effectively controlled IA in vivo and after DLI. Epitopes derived from PRAME, NPM1mut, RHAMM, WT-1 and multiple, life-threatening post-transplant infections. mp-STs contributed to clinical outcome of patients treated with DLI.

E1084 DONOR LYMPHOCYTE INFUSION IN PATIENTS WITH HEMATOLOGICAL MALIGANNCIES LEADS TO DIVERSITY OF LEUKEMIA-ASSOCIATED-ANTIGEN-SPECIFIC T CELL RESPONSES AND TO REDUCTION IN REGULATORY T CELL FREQUENCY
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Background: Cytotoxic T-cell (CTL) responses against malignant cells play a major role in maintaining remission and prolonging overall survival in patients with hematologic malignancies after allogeneic stem cell transplantation (allo-SCT) and/or donor lymphocyte infusions (DLI). Graft versus leukemia (GvL) effects after allogeneic stem cell transplantation and/or DLI are considered to be T cell-mediated. Many groups described specific T-cell responses against several leukemia-associated antigens (LAA) in different hematological malignancies. However, T cell responses after allo-SCT and DLI are not well characterized.

Aims: In this study, we analyzed LAA-specific T cell responses after allo-SCT and DLI. To this end, we assessed the frequency and diversity of LAA-specific CTL responses using ELISpot analysis and tetramer assays in 12 patients (5 patients (pts) with acute myeloid leukemia, 2 pts with chronic myeloid leukemia, 3 pts with multiple myeloma and 2 pts with chronic lymphatic leukemia) before and after DLI. Epitopes derived from PRAME, NPM1mut1, HLA, WT-1 and other LAA were tested. Moreover, the frequency of regulatory T (Treg) cells was measured and the course of cytokine profiles before and after DLI was analyzed. These immunological findings were correlated to the clinical course in the respective patients.

Methods: In ELISpot and tetramer assays, an increase in frequency and diversity of LAA-specific T cells was observed in all patients. Cytokine assays using ELISA for the detection of more than 10 cytokines before and after DLI were employed.

Results: Importantly, there was a significant increase from 0 to 7 LAA-derived T cell epitopes (P<0.03) in clinical responders (R) when compared to non-responders (NR). These positive results in R versus NR where confirmed by tetramer-based flow cytometry assays, where an increase in frequency from 0.5 to 2.3% in the R group of LAA-specific T cell/all CD8+ T cells was observed. Interestingly, the frequency of Tregs in clinical responders decreased significantly from a median 72.9% 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.

Aims: To determine whether NK-92MI cells co-express the CD16 and CD64, whether we were able to enhance their cytotoxicity using CD16 and CD64 antibodies, and whether we could further enhance these killing abilities using a CD64-BB-ζ receptor.

Methods: We generated 23±5x10^7 cells mp-STs (12-fold expansion). All cell lines were polyclonal expressing constitutive receptor memory markers and specific against Asp [SFC/2x10^5 cells: 315±82] and the targeted viruses, if derived from seropositive donors [SFC/2x10^5 cells, CMV: 637±267; EBV: EBNA1, LMP2, BZLF1; BK: Large T, VP1] and Asp pepmixes (Crf1, Ge1, SHMT) and culturing for 10 days. The specificity of mp-STs was analyzed by IFN-γ Elispot. A total of 1.5x10^7 immunomagnetically isolated CD3+cells (donor lymphocyte infusions-DLI) or mp-STs were infused in myelo/immuno-ablated NSG mice which had been intra-nasally inoculated with Asp conidia or left uninfected. Cells were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assessed by histology and immunohistochemistry.

Results: We generated 23±5x10^7 cells mp-STs (12-fold expansion). All cell lines were polyclonal expressing constitutive receptor memory markers and specific against Asp (spot forming cells [SFC]/2x10^5 cells: 315±82) and the targeted viruses, if derived from seropositive donors [SFC/2x10^5 cells, CMV: 637±267; EBV: EBNA1, LMP2, BZLF1; BK: Large T, VP1) and Asp pepmixes (Crf1, Ge1, SHMT) and culturing for 10 days. The specificity of mp-STs was analyzed by IFN-γ Elispot. A total of 1.5x10^7 immunomagnetically isolated CD3+cells (donor lymphocyte infusions-DLI) or mp-STs were infused in myelo/immuno-ablated NSG mice which had been intra-nasally inoculated with Asp conidia or left uninfected. Cells were evaluated by a 5-parameter sickness score and at sacrifice, tissues were assessed by histology and immunohistochemistry.

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.

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-92Mih 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 CD8a extracellular domain, CD28 transmembrane domains, two costimulatory domains (CD28 and 4-1BB), and the signaling domain from CD3ζ. The resulting chimeric receptors, termed CD16-BB-ζ and CD64-BB-ζ, were utilized to generate chimeric receptor-modified NK-92MI cells, which were named NK-92MI-hCD16 and NK-92MI-hCD64 cells, respectively.

Results: We found that NK-92MI-hCD16 and NK-92MI-hCD64 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 BSISPECIFIC 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 acting through the formation of an immunologic synapse between T-cells (CD3) and a tumor-associated surface antigen (TAA) have been used as immunotherapy leading to T-cell activation and viral lysis of tumor cells.

Aims: The aim of the present study is develop and in vitro assay with multiples variables to better quantify the activity of bsippecific antibodies and reflect the interpatient variability.

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 CD123×CD3 (Creative Biolabs) and 7 CLL and 3 B-ALL samples with Blinatumomab (Amgen). When appropriate, basal quantification of TAA was performed by flow cytometry (FCM). The resulting pharmacokinetic parameters were calculated by fitting the responses of BsAbs exposed by each BsAb with the MRL-2 fitting. The results were expressed as EC50 or Emax, even more marked between CLL samples. The integration of effective T:R 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 immunoecheckpoint to unblock this immunoresistant status.

Summary/Conclusions: We have developed an automated flow cytometry assay for bsippec specific antibodies screening that keeps basal quantification of TAA but improved valuable data about the BsAbs activity. The integration of effective T:R ratios 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.

E1087

HUMANIZED CD7 NANOBODY-BASED IMMUNOTOXINS EXHIBIT PROMISING ANTI-T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA POTENTIAL

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Background: Nanobodies, or named as VHHs, are derived from heavy-chain only antibodies that circulate in sera of camels. Their exceptional physicochemical properties, possibility of humanization and unique antigen recognition properties make them excellent candidates for targeting delivery of biologically active components. In our previous efforts, we have successfully generated the monovalent and bivalent CD7 nanobody-based immunotoxins, which can effectively trigger the apoptosis of CD7 positive malignant cells.

Aims: To pursue the possibility of translating these immunotoxins into clinics, we humanized the nanobody sequences (designated as dhuVHH-PE), 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, dhuVHH-PE38, dVHH-PE-LR, and dhuVHH-PE-LR, were successfully constructed. These recombinant immunotoxins were expressed in E. coli and showed that nanobody immunotoxins have the benefits of easy soluble expression in a prokaryotic expression system.

Results: Flow cytometry results revealed that all immunotoxins still maintained their ability to specifically target to CD7-positive T-lymphocytes both in vitro and in vivo. Treatment significantly prolonged mice survival with around 40% survival improvement. However, it is also noticed that despite dhuVHH-PE-LR showed strong anti-tumor effect in vitro, its in vivo anti-tumor efficacy is disappointed.
Results: The cytotoxicity of BCMA against MM cells was determined in vitro and in vivo in a murine MM model; furthermore, their impact in BC-NK cytotoxicity against MM was also determined in vitro. BCMA expression on MM cells after BC-NK exposure was analyzed by confocal microscopy and by flow cytometry. FACs sorting experiments were performed to analyze BCMA transfer between BC-NK exposed MM cells to neighboring non-exposed BC-NK MM cells. Results: Altorvastatin and Fluvastatin treatment (1µM) decreased MM cell line (APR1, 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 BC-NK cytotoxicity against MM cells. Furthermore, pretreatment of MM cells with Fluvastatin (3 µM) increased BC-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, BC-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 BCMA upregulation in MM cells. Furthermore, FACs sorting experiments showed that MM cells exposed to BC-NK, transferred BCMA to neighboring non-BC-NK exposed MM cells which was partially inhibited with Fluvastatin pretreatment. Summary/Conclusions: Our findings show that besides the anti-MM activity of statins alone, they avoid the loss of BCMA expression on MM cells after immune 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 cells.

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 vivo tumoral responses were evaluated. Results: DCs combined with lenalidomide and PD-1 blockade showed the best tumor regression among the study groups. These anti-tumor effects have meaningfully related to the decrease of immuno-regulatory populations, such as myeloid-derived suppressor cells (MDSCs), M2 macrophages, and regulatory T cells (Tregs) and the increase of effector immune cell populations, including CD4+ and CD8+ T cells, natural killer (NK) cells, and M1 macrophages, accompanied with the activation of cytotoxic T lymphocytes (CTLs) and NK cells in the splenocytes from the treated mice. Moreover, the level of immunosuppressive cytokines, such as TGF-β and IL-10, was significantly reduced in tumor microenvironment.

Summary/Conclusions: DC vaccination in combination with lenalidomide plus PD-1 blockade has synergistically induced a strong anti-tumor immunity by modulating tumor microenvironment in a murine myeloma model. This protocol will become a promising translational approach to improve the efficacy of immunotherapy in the field of MM.

E1090

B- AND T-CELL IMMUNE REPertoire PROFILING WITH ANCHORED MULTIPLEX PCR AND NEXT-GENERATION SEQUENCING
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Background: NGS-based analysis of the immune repertoire (IR) is a powerful tool to monitor disease, adaptive immune responses to disease, vaccination and therapeutic interventions. IR characterization by NGS usually requires large primer panels to cover its extensive combinatorial diversity, and a complex system of synthetic controls to account for differential amplification efficiency across segment combinations. Anchored Multiplex PCR (AMP™) uses molecular bar-coded (MBC) adapters and gene-specific primers (GSPs), enabling NGS-based immune chain mRNA interrogation from a single sample. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating dilution and bias due to some adapter saturation.

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 quantitation sensitivity, reproducibility and sensitivity of the IR model. We have successfully constructed a targeted CD79A and CD79B PCR assays using AMP-based NGS in healthy donors, B-cell chronic lymphocytic leukemia donors and formalin-fixed paraffin-embedded (FFPE) tissue.

Results: We developed the AMP-based NGS assays, Immunoverse™ (IGH and TR) for B-cell and T-cell receptor sequencing, respectively. Both assays demonstrated high reproducibility between replicates with quantitative clone tracking down to 0.01%. The ability to determine isotype, clonotype and IGHV mutational status in a single assay was demonstrated. Preliminary TCR assay data indicates that CD3 sequence capture is possible from FFPE tissue with clonotype calling being driven by input quantity, T-cell content, and, to a lesser degree, mRNA quality.

Summary/Conclusions: AMP-based NGS with MBC quantification and error-correction is a powerful method to characterize the immune repertoire.

E1091

SYNERGISTIC ANTITUMOR IMMUNITY BY DENDRITIC CELLS IN COMBINATION WITH POMALIDOMIDE AND DEXAMETHASONE IN A MURINE MYELOMA MODEL
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Background: Pomalidomide (Pom) plus dexamethasone (Dex) could be considered one of the new treatment options in patients with relapsed and/or refractory multiple myeloma (MM). Recently, several diverse agents would be combined to improve the therapeutic efficacy of immunotherapy.

Aims: In this study, we investigated the preclinical efficacy of combined therapy with dendritic cells (DCs) and Pom-Dex in a murine myeloma model.

Methods: After establishing myeloma-bearing mice, four treatment groups were designed to be a mimic protocol as like treatment in clinics as following: 1) PBS control, 2) DCs, 3) Pom + 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 (Tregs) and type 2 macrophages (M2), and the increase of effector cell populations, including activated CD4 T cells, and type 1 macrophages (M1), accompanied with the activation of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells in the splenocytes from vaccinated mice.

Conclusion: Our findings show that combined therapy of the DC combined with Pom and DexA synergistically enhance the anti-tumor immunity in a murine myeloma model, by skewing immuno-suppressive status toward immuno-suppressive status in tumor microenvironment.
E1092

ALTERATIONS IN T-CELL SUBPOPULATIONS AFTER CO-CULTURING WITH MSCS DERIVED FROM DIFFERENT DONORS

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Background: Study of interactions between lymphocytes and mesenchymal stromal cells (MSCs) in vitro revealed increase of HLA-DR expression on T-cells after co-cultivation with some MSCs samples. On lymphocytes derived from one donor the elevation of HLA-DR was observed after co-cultivation with half of MSCs samples (group A). On the other the HLA-DR expression level did not change (group B). MSCs were divided into two groups based on HLA-DR rise on lymphocytes. Study of T-cell subpopulations after interactions with MSCs could explain ineffectiveness of some MSCs as an immunomodulating agent in clinical applications.

Aims: The aim of the study was to discriminate variations in T-cell subpopulations, co-cultured with MSCs from two groups.

Methods: MSCs were isolated from bone marrow of 13 donors for allogeneic hematopoietic cells transplantation and cultured by a standard method. MSCs were seeded 10^5 cells per flask, and then 10^4 allogeneic lymphocytes from single donor were added to all MSCs cultures. For lymphocytes activation 5mg/ml phytohemagglutinin (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 and PD-1 were studied by flow cytometry as well as distribution of naïve and effector T-cells were analyzed on 4th day of cultivation. p<0.05 was considered statistically significant; all data are presented as medium ± SEM.

Table 1.

Results: Expression of HLA-DR on lymphocytes after 4 days of cultivation without MSCs did not change compared to 1st day. When lymphocytes were co-cultured with some MSCs samples expression of HLA-DR was higher. Elevated percentage of HLA-DR positive cells correlates between CD4+ and CD8+ cells (R^2=0.932). Thus samples of MSCs were divided into two groups: in group A proportion of HLA-DR lymphocytes was 3 times greater than in group B. Subpopulations of lymphocytes co-cultured with MSCs from group A and B were compared. Subpopulations which significantly differed between groups A and B are presented in the table. In lymphocytes co-cultured with MSCs there were higher number of 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 cultures without MSCs, p<0.001). Group B showed lower number of EM and TM cells. Differences between groups were more pronounced when lymphocytes were activated. In group B proportion of HLA-DR CD4+ and CD8+ cells was significantly lower, compared to group A and control samples. At the same time the number of CM and PD-1+ CD4+ cells was lower in group B, but number of TE was increased. Investigation of HLA-DR expression on MSC after co-culturing with lymphocytes showed higher level of fluorescence signal (MFI) in group A then group B (635±130 vs 289±18, p=0.03). These data indicated that MSCs from group A had become more immunogenic after interaction with lymphocytes and could not show immunomodulating properties in same way as MSCs from group B.

Summary/Conclusions: The immunomodulatory properties of MSCs depend on the donor. This could explain why administration of MSCs is not always successful. Preliminary study of MSCs prior to their administration may be used to predict their efficiency in the future.

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E1093

GRANULOCYTE COLONY STIMULATING FACTOR AND ERYTHROPOIETIN ENTERTALLY GIVEN FOR NEONATES RECOVERING FROM GIT SURGERIES: RANDOMIZED CONTROLLED TRIAL

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Background: Feeding intolerance is a common problem among neonates recovering from surgery for congenital abnormalities of the gastrointestinal tract (GIT) such as small bowel atresia, omphalocele or gastroschisis. Feeding intolerance is a multifactorial process, but one of the important reasons is congenital maldevelopment of the small bowel villi. Disuse atrophy of the small bowel mucosa following several days of post-operative enteral fasting is one factor that can contribute to feeding intolerance. The human fetus swallows over 200 ml/kg/day of amniotic fluid and such swallowing is essential for normal small bowel development. Growth factors found in the amniotic fluid have been shown to promote proliferation of fetal intestinal cells. These growth factors include epidermal growth factor, granulocyte colony stimulating factor (G-CSF) and erythropoietin (EPO). We postulated that infants recovering from surgeries for congenital obstructive bowel abnormalities could be provided with physiologic quantities of recombinant human G-CSF and EPO by the intermittent orogastric or nasogastric administration of 20 ml/kg/day of sterile isotonic solution that contained cytokine concentrations comparable to what they would have ingested from amniotic fluid in utero.

Aims: To test a hypothesis suggesting that feeding tolerance could be improved in neonates recovering from surgeries for congenital obstructive bowel abnormalities by enterally administering recombinant human G-CSF and EPO included within simultaneously administered sterile saline solution.

Methods: This double-blinded randomized controlled clinical trial was conducted on 40 neonates recovering from GIT surgeries for congenital bowel abnormalities. Hemodynamically unstable babies, and those with any contraindication to enteral feeding were excluded. Neonates were randomly divided postoperatively into 2 groups; 20 neonates received the test solution (called Simulated Amniotic Fluid-like solution given Enterally; SAFE); 20 neonates enterally received distilled water (control). Treatment was started postoperative and the test solution (or distilled water) was discontinued when enteral intake reached 100cc/kg/day. Feeding tolerance and adverse effects of treatment (if any) were assessed.

Results: All the studied neonates tolerated the received solution well without side effects that could be attributed to its intake. The study group showed better feeding tolerance as reflected by earlier achievement of 50, 100, 120 and full enteral feeds with higher enteral caloric intake 7 days after SAFE administration and higher rate of weight gain (p<0.05). No significant increase was found in the level of WBCs count, hemoglobin and hematocrit values either pre-initiation or 7 days after administration of SAFE (p>0.05).

Summary/Conclusions: This study provides further insights on the improve ment of neonatal outcomes and help to decrease morbidities from post-operative malnutrition and feeding intolerance. Enteral administration of rhG-CSF and rhEPO may play a critical role in preventing villous atrophy, thereby, reducing feeding intolerance in neonates recovering from surgeries for congenital bowel abnormalities.

E1094

GENE EDITING OF HUMAN HEMATopoietic PROGENIToRs TO CORRECT PRyvUATE KINESE DEFICIENCY

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Background: Pyruvate Kinase Deficiency (PKD) is a rare erythroid metabolic disease caused by mutations in the PKLR gene which encodes the erythroid specific Pyruvate Kinase (RPK) enzyme. The defective enzyme fails to produce normal ATP levels and consequently, erythrocytes from PKD patients show an energetic imbalance and are susceptible to hemolysis. Site-specific hematopoietic stem cell gene therapy would be the safest approach to treat PKD patients. In this study, different gene editing approaches have been explored to correct PKD, either by the Knock-in of a PKLR cDNA sequence in the second intron of PKLRF1 or by the site-directed correction of specific mutations.

Aims: In the Knock-in system, that previously showed to correct the PKD phenotype of PKD-iPSC lines, a recombination matrix carrying codon optimized exons 3-11 of the PKLR cDNA sequence in the second intron of PKLR, either by the Knock-in of a PKLR cDNA sequence in the second intron of PKLRF1 or by the site-directed correction of specific mutations.

Methods: Thus, the therapeutic matrix together with specific TALENS as DNA plasmid or mRNA, for the second exon of PKLR were electroporated in purified CD34+ cells from healthy cord bloods. Cells were then expanded and puromycin selected to enrich the population for gene edited ones.
E1095 BLAST KINETICS AFTER NON-ENGRAFTING HAPLOIDENTICAL MICROTRANSPLANTATION IN PATIENTS WITH REFRACTORY ACUTE MYELOID LEUKAEMIA

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Background: Multiple trials have showed that granulocyte colony-stimulating factor (G-CSF)-mobilized donor peripheral-blood stem cells (GPBSCs) based on allo-graft can be effective in mediating graft-versus-leukemia (GVL) effects and promote hematologic recovery without triggering of acute GVHD.

Aims: To analyze the safety and efficacy of non-engraftment haploidentical cellular therapy for patients with refractory acute myeloid leukaemia 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 leukaemia were enrolled into this Phase I/II study. They were treated with chemotherapy including fludarabine 30mg/m2, cytarabine 1gm/m2, and melphalan 140mg/m2 plus busulfan 16mg/m2. Three patients (2 males 80%, 1 female 20%) were transplanted at day +50 from the 1st microtransplantation and at day +100 (day +75 from the 2nd microtransplantation). Three patients (2 males 66.6%, 1 female 33.3%) were transplanted at day +60 from the 1st microtransplantation (day +30 from 2nd microtransplantation). The cellular therapy was performed using unmanipulated GPBSCs 24 hour after last chemotherapy infusion. Morphologic assessment of bone marrow blast kinetic by bone marrow aspiration conducted before therapy, D14 and D30 after therapy. Hematopoietic cells percent kinetics (Hematologic recovery) was assessed by complete blood count every day till Day 40.

Results: At day +30, 6 patients were evaluable for response and one patient had died. One patient out of 7 showed PR, then developed CR after a second microtransplantation. Seven patients (4 males 57.1%, 3 females 42.9%) with refractory acute myeloid leukaemia were transplanted into this Phase I/II study. They were treated with chemotherapy including fludarabine 30mg/m2, cytarabine 1gm/m2, and melphalan 140mg/m2 plus busulfan 16mg/m2. Three patients (2 males 80%, 1 female 20%) were transplanted at day +50 from the 1st microtransplantation and at day +100 (day +75 from the 2nd microtransplantation). Three patients (2 males 66.6%, 1 female 33.3%) were transplanted at day +60 from the 1st microtransplantation (day +30 from 2nd microtransplantation). The cellular therapy was performed using 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.

Aim: The aim of the study was to determine the distribution of naive and effector cells in lymphocytes co-cultured with MSCs.

Methods: MSCs were derived from bone marrow of 13 donors (7 male and 6 female aged 22 to 62 years, median 27 years). MSCs were co-cultured with 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 IFN-γ (mMSCs). Activation markers CD25, CD38, CD69, HLA-DR and PD-1 were studied by flow cytometry and the distribution of naive and effector T-cells in T-cells were analyzed.

Results: By the fourth day of incubation the proportion of naive CD4+ cells reduced by 30% (from 47.5±3.0% to 32.8±3.3%) and cellularized lymphocytes. It did not happen in lymphocytes co-cultured with MSCs and mMSCs (p=0.001). At the same time in cultured lymphocytes to the fourth day 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 mMSCS (p=0.001). Thus, co-culturing with MSCS or mMSCS prevented naive T-lymphocytes transiting into effector cells. The proportion of CD4+PD-1+ cells increased from 8.2±1.1% to 10.9±0.7% by the 4th day of cultivation. When co-cultured with MSCS and mMSCS the proportion of CD4+ cells did not change (p=0.0125). The proportion of HLA-DR+ on both CD4+ and CD8+ cells in lymphocytes remained unchanged for 4 days. When co-cultured with MSCS and mMSCS for 4 days there was a consistent increase in the proportion of CD4+/HLA-DR+ (e.g. 10.1±1% to 15.6±1.1%, p=0.005) and CD8+/HLA-DR+ (from 9.7±0.8% to 26.0±3.7%, p=0.024). So allogeneic MSCS induced peptide presentation on lymphocytes. The proportion of CD4+ central memory cells increased in PHA-lymphocytes from 37.4±4.4 at 1st day to 68.2±6.5 at 4th day. MSCS inhibited this increase - the proportion CD4+ central memory cells increased from 24.4±2.7% to 46.6±4.5% (p=0.047). Thus the interaction of PHA-lymphocytes with MSCS inhibited their activation and preserved naive state.

Summary/Conclusions: The composition of lymphocyte population changes during cultivation. The proportion of naive cells reduced, while the number of effector cells and the proportion of PD-1+ increased, indicating lymphocyte activation probably due to the presence of xenogeneic serum in the culture medium. Co-cultivation with MSCS maintained lymphocytes in not activated state. The interaction of activated lymphocytes with MSCS inhibits their activation and preserves naive state. IFN-γ priming did not enhance MSCS inhibitory effect. Lymphocyte activation showed that MSCS both preserve naive lymphocyte condition and have an inhibitory effect on their activation.

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The materials, supported by grant from the Russian Science Foundation, Project № 16-15-00102.

E1096

INTERACTION OF MULTIPOTENT MESENCHYMAL STROMAL CELLS WITH LYMPHOCYTES REDUCES THEIR IMMUNO PRIVILEGED PROPERTY

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Aims: To determine the precise window of specification of HE hematopoietic precursors and interrogate at the single cell level their functional heterogeneity.

Methods: Dams pregnant with Cdh5ERT2-CreROSA26Confetti/+ embryos were treated with tamoxifen (TAM) at E7.5, E8.5, E9.5, E10.0 or E11.5. Here, TAM induces the permanent and random labeling of endothelial cells and their progeny with one of the Confetti allele fluorescence reporters (YFP, GFP, CFP or RFP). The blood of resulting adult Cdh5ERT2-CreROSA26Confetti/+ offspring was then examined for the presence of Confetti+ cells by flow cytometry. Clonal ex vivo assays: VE-Cadherin+CD45- cells, which contain HE, were isolated by FACs from E9.5, E10.5 or E11.5 embryos and co-cultured at limiting dilution either with or without PHA-activated lymphocytes. HE and HE-derived endothelial cells engineered to express Myr-AKT (AA-ECS), which both support hematopoietic output from HE ex vivo. Co-cultures were scored for hematopoietic colony formation, which were then analyzed for hematopoietic re-plating activity and flow cytometry for hematopoietic cell surface markers.

Results: To estimate the temporal window of TAM activity, CD45.2+ROSA26ERT2-Cre/Confetti+ bone marrow (BM) cells were transplanted into CD45.2+CD45.1+ recipients treated with TAM three, two, one or zero days before transplant. Only the PB of recipients treated on the same day of transplant showed 100% CoC and Confetti+cells. HE recruitment was observed in PB of recipients treated with TAM 2, 1 or 0 days before transplant. TAM-induced HE recruitment was not observed in recipients treated with TAM 3 days before transplant. The only observed TAM-induced recruitment was detected in recipients treated with TAM 2 days before transplant. The results show that the window of TAM activity is approximately 24 hours. Analysis of 10 weeks old Cdh5ERT2-CreROSA26Confetti+ mice revealed that only mice exposed to TAM in utero at E8.5 and E9.5 had Confetti+ blood. Thus, specification of HE begins at E8.5 and is complete by E10.5. Next, E11.5 AGMs isolated from CD45.2+ Cdh5ERT2-CreROSA26Confetti+ embryos exposed to TAM at E10.5 were cultured as explants for three days under conditions that preserve ongoing HSC specification from HE, dissociated, and then transplanted into CD45.2+CD45.1+ recipients. Remarkably, although the CD45.2+ chimera was (~80%) in the blood of recipients, all CD45.2+ blood was negative for the Confetti label, further indicating that HE recruitment is complete by E10.5 and cannot be reactivated during explant culture. Limiting dilution culture of E9.5, E10.5, and E11.5 VE-Cadherin+CD45- endothelium revealed the frequency of functional HE to be 0.1, 1.1 and 0.19% at these time-points, respectively. Phenotypic analysis of primary hematopoietic colonies revealed cells expressing the C-type lectin-like receptor 2 (CLEC2) and the only a small population of colonies producing phenotypic HSCs in AA-ECS co-cultures. These data suggest the presence of HE precursors with distinct functional output or the existence of a continuum of HE at different stages of maturation.

Summary/Conclusions: We have defined the window of mammalian HE specification. The abrupt loss of ongoing HE recruitment at E10.25 suggests an active mechanism that terminates this process. We also observed large phenotypic and functional variability amongst individual HE precursors examined throughout ontogeny.

E1100

C-TYPE LECTIN-LIKE RECEPTOR 2 SPECIFIES A FUNCTIONALLY DISTINCT SUBPOPULATION OF MEGAKARYOCYTE-BASED LONG-TERM HEMATOPOIETIC STEM CELLS

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Background: Hematopoietic stem cells (HSCs) arise from hematopoietic endothelial (HE) precursors between embryonic day 10.5 (E10.5) and 12.5 of murine development, primarily in the aorta-gonad-mesonephros (AGM) region and the umbilical (UA) and vitelline arteries (VA). The window of specification of HE has not been defined in any mammalian system.

Aims: To determine the precise window of specification of HE hematopoietic precursors and interrogate at the single cell level their functional heterogeneity.

Methods: Dams pregnant with Cdh5ERT2-CreROSA26Confetti/+ embryos were treated with tamoxifen (TAM) at E7.5, E8.5, E9.5, E10.0 or E11.5. Here, TAM induces the permanent and random labeling of endothelial cells and their progeny with one of the Confetti allele fluorescence reporters (YFP, GFP, CFP or RFP). The blood of resulting adult Cdh5ERT2-CreROSA26Confetti/+ offspring was then examined for the presence of Confetti+ cells by flow cytometry. Clonal ex vivo assays: VE-Cadherin+CD45- cells, which contain HE, were isolated by FACs from E9.5, E10.5 or E11.5 embryos and co-cultured at limiting dilution either with or without PHA-activated lymphocytes. HE and HE-derived endothelial cells engineered to express Myr-AKT (AA-ECS), which both support hematopoietic output from HE ex vivo. Co-cultures were scored for hematopoietic colony formation, which were then analyzed for hematopoietic re-plating activity and flow cytometry for hematopoietic cell surface markers.

Results: To estimate the temporal window of TAM activity, CD45.2+ROSA26ERT2-Cre/Confetti+ bone marrow (BM) cells were transplanted into CD45.2+CD45.1+ recipients treated with TAM three, two, one or zero days before transplant. Only the PB of recipients treated on the same day of transplant showed 100% CoC and Confetti+cells. HE recruitment was observed in PB of recipients treated with TAM 2, 1 or 0 days before transplant. TAM-induced HE recruitment was not observed in recipients treated with TAM 3 days before transplant. The only observed TAM-induced recruitment was detected in recipients treated with TAM 2 days before transplant. The results show that the window of TAM activity is approximately 24 hours. Analysis of 10 weeks old Cdh5ERT2-CreROSA26Confetti+ mice revealed that only mice exposed to TAM in utero at E8.5 and E9.5 had Confetti+ blood. Thus, specification of HE begins at E8.5 and is complete by E10.5. Next, E11.5 AGMs isolated from CD45.2+ Cdh5ERT2-CreROSA26Confetti+ embryos exposed to TAM at E10.5 were cultured as explants for three days under conditions that preserve ongoing HSC specification from HE, dissociated, and then transplanted into CD45.2+CD45.1+ recipients. Remarkably, although the CD45.2+ chimera was (~80%) in the blood of recipients, all CD45.2+ blood was negative for the Confetti label, further indicating that HE recruitment is complete by E10.5 and cannot be reactivated during explant culture. Limiting dilution culture of E9.5, E10.5, and E11.5 VE-Cadherin+CD45- endothelium revealed the frequency of functional HE to be 0.1, 1.1 and 0.19% at these time-points, respectively. Phenotypic analysis of primary hematopoietic colonies revealed cells expressing the C-type lectin-like receptor 2 (CLEC2) and the only a small population of colonies producing phenotypic HSCs in AA-ECS co-cultures. These data suggest the presence of HE precursors with distinct functional output or the existence of a continuum of HE at different stages of maturation.

Summary/Conclusions: We have defined the window of mammalian HE specification. The abrupt loss of ongoing HE recruitment at E10.25 suggests an active mechanism that terminates this process. We also observed large phenotypic and functional variability amongst individual HE precursors examined throughout ontogeny.

E1099

SPECIFICATION OF MURINE HEMOGONIC ENDOTHELIAL HEMATOPOIETIC PRECURSORS CEASES ABRUPTLY BY E10.25 AND CONSTITUTES A FUNCTIONALLY HETEROGENEOUS POPULATION

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Background: Recent studies have supported the model in which hematopoietic stem cell (HSC) compartment consists of functionally distinct subsets with discrete self-renewal and differentiation potentials. However, their immunophenotypes and the functional diversities remain poorly understood. We previously reported that the authentically identified HSC population includes a subset of cells expressing the C-type lectin-like receptor 2 (CLEC2) that are able to give rise to megakaryocyte progenitors (MKPs) and megakaryocytes bypassing the pathway from common myeloid progenitor (CMP) to megakaryocyte/erythrocyte progenitor (MEP) (21th Congress of EHA, # P356, 2016).
Aims: In this study, we analyzed in vivo dynamics of CLEC2high HSCs to clarify their functional roles in adult hematopoiesis.

Methods: In this experiment, we defined Lin−Sca1+Kit+CD150+CD34− cells as HSCs and Lin−Sca1+Kit+CD150+CD41+ as MP. We performed transplantation assays using HSCs isolated from EGFP transgenic (CAG-EGFP+) mice to trace donor-derived HSCs and their progeny. Consequently, excepting enucleated donor-derived Mpk, CLE2high HSCs and CLEC2low HSCs were transplanted into lethally irradiated mice, respectively. Chimerism and lineage distribution of donor-derived cells were evaluated periodically by tracing EGFP. Secondary transplantation was performed by transferring 1x107 BM cells from the recipient mice 16 weeks after the 1st transplantation.

Results: Bone marrow analysis revealed that both EGFP+CLEC2high and CLEC2low donor-derived HSC populations were detected for up to 12 weeks after transplantation. Also, these subsets were capable of generating all lineages of cells in transplanted mice. Interestingly, CLEC2high HSCs generated CLEC2high donor-derived HSCs already observed in the steady-state BM, vice versa. Consistent with these reciprocal transition, both types of HSCs could effectively reconstitute hematopoiesis in the secondary recipients. However, CLEC2high HSCs showed significantly reduced repopulating activity than CLEC2low cells, especially at 12 weeks after transplantation (mean of EGFP+ HSC proportion in the primary recipients with CLEC2high HSCs vs CLEC2low HSCs: (each 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 CLEC2low HSCs kept high chimeric levels of EGFP+ CMP and MEP, while these levels decreased in the recipients with CLEC2high HSCs. On the other hand, CLEC2high HSCs yielded 2.5-fold more Mpk than CLEC2low HSCs in short-term grafts (1 to 2 weeks after transplantation) (p<0.05). Consistent with this finding, CLEC2high HSCs yielded more CD41+ platelets than CLEC2low HSCs by 6.0-fold at 1 week after transplantation (p<0.05), which peaked 10 weeks earlier than in CLEC2low recipient mice. These platelets yielded through the transplantation of CLEC2high HSCs were rare during 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 CLEC2high recipients, indicating that CLEC2 signaling is essential for rapid and enhanced megakaryopoiesis from CLEC2high HSCs.

Summary/Conclusions: Here, we showed that CLEC2 expression on HSCs demonstrates their oscillation for serving as a potent source of megakaryopoiesis, and found that CLEC2/Syk signaling would be involved in differential regulation between CLEC2high and CLEC2low HSC subtypes.

E1101
PRE-TRANSPLANT DEFECTS OF BONE MARROW ENDOTHELIAL CELLS MAY CAUSE THE OCCURRENCE OF POOR GRAFT FUNCTION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: Poor graft function (PGF) is a serious complication after allogeneic hematopoietic stem cell transplantation (allo-HSCT). PGF is defined as complete donor hematological chimerism with no residual or recurrent leukemia, but a hypo- or aplastic bone marrow (BM) with 2 or 3 of the following: (1) neutrophils <0.5 x 10^{9}/L; (2) platelets <20 x 10^{9}/L; and/or (3) hemoglobin concentration <0.10 on univariate logistic regression analysis between PGF and pre-HSCT, significantly reduced percent- age of BM EPCs were observed in PGF patients than those in GGF patients, whereas no significant differences were found in the percentage of BM HSCs and their progeny. Moreover, similar ROS levels were demonstrated in BM EPCs and HSCs between PGF and GGF patients. Although there was no difference in transplanted CD34+ cell dose between the PGF and GGF groups, significantly lower percentages of BM EPCs and HSCs, whereas remarkably higher ROS levels were observed in BM EPCs and HSCs in PGF recipients than those in GGF patients. Moreover, inverse correlations were observed between BM EPCs frequency and their ROS levels post-HSCT, as well as BM HSCs frequency and their ROS levels post-HSCT. Multivariate analyses revealed that the reduced BM EPCs and the disease status pre-HSCT were independent risk factors for the occurrence of PGF following allo-HSCT.

Summary/Conclusions: We identified that patients with impaired BM EPCs pre-transplant were at a high risk for the occurrence of PGF post-allo-transplant. Moreover, persistent low percentage and high levels of ROS in BM EPCs may hamper the hematopoietic reconstitution of engrafted donor HSCs in patients with PGF. Therefore, strategies to repair the impaired BM EPCs appear to be a promising therapeutic approach in patients with PGF after allo-HSCT.

E1102
EFFICIENT LYMPHOID DIFFERENTIATION OF HEMATOPOIETIC STEM CELLS REQUIRES CXCR4 DESENSITIZATION

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Background: The Warts, Hypogammaglobulinemia Infections and Myelokathexis Syndrome (WS) is a rare immunohematological disorder characterized notably by a chronic lymphopenia. It is mostly caused by inherited heterozygous autosomal gain-of-function mutations in CXCR4, which enforces a distal truncation in the C-terminal domain and leads to a desensitization-resistant receptor. Given that CXCR4 is widely expressed on non-hematopoietic cells and virtually on all leukocytes at multiple stages of development, one possibility could be that the WS-associated peripheral blood lymphopenia is a consequence of skewed production, differentiation or distribution of lymphocytes related to altered CXCR4-mediated signaling. Recently, we have been able to replicate the hematologic phenotype of WS using a knock-in mouse strain that harbors the WS-linked heterozygous CXCR4S338X mutation causing a distal truncation of the last 15 residues of the C-terminal domain (Balabanian et al., Blood, 2012). Mutant mice displayed lymphocytes with enhanced migration to Ccxl2, phagocytosed severe lymphopenia and failed to maintain antibody titers after immunization (Biajoux et al., Cell Reports, 2016). First-line analyses of 1x10^13 mice suggested developmental defects at the pro/pre-B cell stage in the bone marrow (BM) and during the early double-negative stages of thymocyte maturation. However, whether impaired lymphopoiesis stems from an upstream cell-intrinsic or the hematopoietic defect remains to be established.

Aims: We took advantage of our relevant knock-in model and the access to blood samples from WS patients to investigate the impact of CXCR4 desensitization on BM and extra-medullary splenic hematopoiesis and recirculation of lymphoid precursors (HSPCs).

Methods: The global hematopoietic development, including quiescence, cycling and survival properties of HSPCs, was examined in non-manipulated and BM-chimeric mice using flow-cytometric and clonogenic-based assays. Cxcr4 expression and function were assessed using internalization, transgenic in vivo homing and AMD3100-promoted mobilization experiments. Both multipotency and survival properties of HSPCs have been assessed using serial BM transplantation experiments. Immunophenotypic and clonogenic analyses of HSPCs were performed from blood samples of five WS patients and age-, sex-, matched healthy controls. Furthermore, the hematopoietic defect remains to be established.

Aims: To investigate whether the BM HSCs in subjects with PGF are impaired pre-HSCT. To compare the reconstitution kinetics of BM HSPCs, their ROS levels in subjects with PGF and good graft function (GFF) post-HSCT.

Methods: A total of 115 patients who will receive allo-HSCT were prospectively recruited, and randomly selected as training group (n=32) and validation group (n=83). The percentage of BM CD45-CD34+VEGF+2+ EPCs, CD34+ HSCs, and reactive oxygen species (ROS) levels in EPCs and HSCs were evaluated in all of the enrolled patients pre-HSCT by flow cytometry. Furthermore, 59 patients were monitored for the frequency and ROS levels of BM EPCs and HSCs by flow cytometry. In order to identify risk factors for PGF, pre-HSCT risk factors with a P<0.10 on univariate logistic analysis were included in the multivariate logistic regression analysis, and factors with a P<0.05 were considered independently associated with PGF.

Results: A total of 18 patients including 5 patients in training group (15.63%) and 13 subjects in validation group (15.67%) developed PGF post-HSCT. Both in training group and validation group pre-HSCT, significantly reduced percentage of BM EPCs were observed in PGF patients than those in GGF patients, whereas no significant differences were found in the percentage of BM HSCs and their progeny. Moreover, similar ROS levels were demonstrated in BM EPCs and HSCs between PGF and GGF patients. Although there was no difference in transplanted CD34+ cell dose between the PGF and GGF groups, significantly lower percentages of BM EPCs and HSCs, whereas remarkably higher ROS levels were observed in BM EPCs and HSCs in PGF recipients than those in GGF patients. Moreover, inverse correlations were observed between BM EPCs frequency and their ROS levels post-HSCT, as well as BM HSCs frequency and their ROS levels post-HSCT. Multivariate analyses revealed that the reduced BM EPCs and the disease status pre-HSCT were independent risk factors for the occurrence of PGF following allo-HSCT.

Summary/Conclusions: We identified that patients with impaired BM EPCs pre-transplant were at a high risk for the occurrence of PGF post-allo-transplant. Moreover, persistent low percentage and high levels of ROS in BM EPCs may hamper the hematopoietic reconstitution of engrafted donor HSCs in patients with PGF. Therefore, strategies to repair the impaired BM EPCs appear to be a promising therapeutic approach in patients with PGF after allo-HSCT.

E1103
A SUBSET OF ADULT HSC DERIVES FROM GATA4-EXPRESSING LMPPS LOCATED IN THE PLACENTA AND LATERAL MESODERM OF MICE

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1Madrid, Spain, June 22 – 25, 2017
E1104

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Background: GATA4 is a transcription factor expressed in mesoderm and endoderm during development. Members of the family such as GATA-1-3, but not GATA-4, are critically involved in hematopoiesis. An enhancer (G2) of the mouse Gata-4 gene directs its expression throughout the lateral mesoderm and the allantois, beginning at E7.5, becoming restricted to the septum transversum by E10.5, and disappearing by midgestation (Rojas et al., Development, 2005, 132:3405). Our previous work has shown that inactivation of Gata-4 using this G2Cre driver is lethal by midgestation (Delgado et al., Development, 2014, 59:2358). The anemia observed in the G2Cre-;Gata-diofluor+ 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;R26REYFP 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-Gata4Cre;R26REYFP mice, adults and embryos, by flow cytometry, RT-PCR and confocal microscopy. Cells obtained from different tissues were cultured and transplanted to analyze in vitro and in vivo potential.

Results: YFP+ cells represented about 20% of the hematopoietic system of adult mice and contributed in the same proportion to the lymphoid, myeloid and erythroid lineages. Adult YFP+ hematopoietic stem cells (Figure 1) constituted a long-term repopulating, transplantable population. Fetal YFP+ hematopoietic progenitors were much more abundant in the placenta than in the fetal liver. These placental YFP+ progenitors were clonogenic in the MethoCult assay and fully reconstituted hematopoiesis in adult mice and contributed in the same proportion to the lymphoid, myeloid and erythroid lineages.

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 embryonic (placental) origin. Both lineages basically showed similar physiological behavior in normal mice, but this finding raises a number of questions, for example: Does this hematopoietic stem cell subpopulation show a different response in physiopathological conditions? Does this subpopulation show a different profile of gene expression? Does a similar heterogeneity exist in human HSCs? We are currently investigating the transcriptome of the G2-GATA4 lineage HSC in order to answer these questions.

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-zinc finger (KRAB-ZNF) transcription factors, has an isoform exclusively expressed in CD34+ hematopoietic stem cell 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 cell viability, homing 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.

Figure 1. The side population of the bone marrow from G2-Gata4Cre;R26REYFP mice identified by Hoechst 2224 staining, which contains adult HSCs, includes a fraction of YFP+ cells. (From Cafeite et al., 2017).

Summary/Conclusions: A lineage of adult hematopoietic stem cells in mice is characterized by the expression of GATA4 in their embryonic progenitors and probably by its embryonic (placental) origin. Both lineages basically showed similar physiological behavior in normal mice, but this finding raises a number of questions, for example: Does this hematopoietic stem cell subpopulation show a different response in physiopathological conditions? Does this subpopulation show a different profile of gene expression? Does a similar heterogeneity exist in human HSCs? We are currently investigating the transcriptome of the G2-GATA4 lineage HSC in order to answer these questions.

E1105

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Aims: We studied the effects of SZF1/ZNF589 overexpression in vitro and evaluated its tumor suppressor potential in vivo.

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-zinc finger (KRAB-ZNF) transcription factors, has an isoform exclusively expressed in CD34+ hematopoietic stem cell 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 cell viability, homing and survival, known to be influenced by the hypoxic state in the bone marrow niche.

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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/ZNFS89 cells were directly injected into the femurs of NSG mice and tumor development was monitored by bioluminescence. Furthermore, K562 cells with or without SZF1/ZNFS89 overexpression were studied by proliferation assay, cytomorphology, 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/ZNFS89 in vivo. In vitro, overexpression of SZF1/ZNFS89 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 assays, these cells were entering premature senescence.

Summary/Conclusions: SZF1/ZNFS89 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 DNAm patterns. It is conceivable that this can at least partly be attributed to alternative splicing of DNMT3A.

Aims: In this study, we followed the hypothesis that specific splice variants of DNMT3A impact on hematopoietic differentiation or DNAm patterns. Therefore we addressed the role of specific splice variants of DNMT3A in hematopoietic stem and progenitor cells (HSPCs).

Methods: Expression of DNMT3A splice variants was modulated in HSPCs: transcript 1+3 (Tr.1+3), transcript 2 (Tr.2), or transcript 4 (Tr.4) of DNMT3A were either knocked down by short hairpin RNA or constitutively overexpressed by lentiviral infection. Expression changes were validated by qRT-PCR. Subsequently, we evaluated the impact on colony formation potential (CFU assay), proliferation (CFSE assay), and the immunophenotype (CD34+ and CD133+). Global DNAm profiles were generated with the Infinium HumanMethylation450 BeadChip platform and gene expression profiles with the Human Affymetrix GeneChip GT1 1.0 platform.

Results: Downregulation of either Tr.2 or Tr.4 reduced the proliferation rate of HSPCs significantly (n=3, p<0.05). HSPCs maintained CD34 expression for a higher number of cell divisions upon knockdown of Tr.2 (n=3, p<0.05). In colony forming unit (CFU) assays downregulation of Tr.4 resulted in a clear bias towards erythroid colonies (n=3, p<0.05). Overall, CFU frequency was reduced by knockdown of DNMT3A transcripts, whereas it was increased by overexpression. Subsequently, we analyzed the impact of specific DNMT3A variants on the DNAm patterns: several CpG sites revealed significant differences in DNAm levels upon knockdown of Tr.2 and Tr.1+3 (8,905 and 352 CpGs, respectively; n=3, adjusted p-value <0.05). Notably, these patterns were regulated in the opposite direction upon overexpression of the same transcripts. Knockdown of Tr.4, which does not have the DNA-methyltransferase domain, did not evoke significant changes in DNAm. Furthermore, modulation of DNMT3A splice variants resulted in transcript-specific gene expression changes, which may at least partly be attributed to the 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.

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 macrophage-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 included 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, 5-12-week-old female mice in accordance with the approval of the Institutional Animal Care and Use Committee of Tel-Aviv University (M-14-043). BM cells were flushed from femurs, tibiae, and pelvic bone and red blood cells were lysed. Cells were stained with labelled anti-mouse antibodies: PE-B220, FITC-CD19, PerCP-igm, PeCy7-CD43, and APC-M-CSF receptor/CD115; and sorted by flow cytometry. Cells were then cultured in α-MEM containing 10% fetal bovine serum, M-CSF and RANKL. Multinucleated osteoclasts were stained for tartrate-resistant acid phosphatase (TRAP) and pit resorption was assessed.

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 EYPF, thus tracing back their B cell origin (Figure 1A).

Next, we dissected which B cell progenitor subtype possesses this osteoclastogenic capacity and found that only Pro-B (B220+CD19+CD43HighIgM-), but not Pre-B (B220+CD19+CD43lowCD115+), nor immature B cells (B220+CD19+CD43IgMhi) could transdifferentiate into osteoclasts (16%±3.7 vs. 0.79%±0.28 and 0.48%±0.13 osteoclasts’ area, respectively). Moreover, among the Pro-B cells, only those expressing M-CSF receptor (CD115) could transdifferentiate into functional osteoclasts (18%±5.5 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) Trans-differentiation of 180,000 cells/well CD19-Cre;R26R-EYFP into osteoclasts. (B) TRAP staining of osteoclasts derived from indicated sorted cells from BM (10,000 cells/well) and cultured with M-CSF and RANKL. Left – Pro-B cells expressing CD115 (B220+CD19+CD43hiIgMhiCD115+). Right – Pre-B cells not expressing CD115 (B220+CD19+CD43IgMhiCD115+). 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 sorted Pro-B cells (B220+CD19+CD43hiIgMhiCD115+). Data are means±SEM of osteoclast area, n=5 mice in each group; *p<0.05.
BONE MARROW MYELOPOIESIS INDEPENDENTLY OF CANONICAL NOTCH SIGNALING

**Background:** Notch signaling is a highly conserved pathway important in multiple developmental processes. Canonical signaling through all Notch receptors converges on the CSL transcription factor recombination signal binding protein for immunoglobulin kappa J region (Rbpj). In haematopoiesis, Notch is critical for the development of the haematopoietic stem cells (HSCs) in the embryo and in thymic T cell development. Contrastingly, canonical Notch signaling has been shown to be dispensable for HSC homeostasis in the adult bone marrow (aBM). Recent studies have however suggested a role of Notch in promoting megakaryocyte (Mk) and 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 other than Notch signaling. Moreover, whether dual inhibition of Notch1 and 2 could ever affect the normal lifespan of mice has never been addressed.

**Aims:** To unambiguously investigate the role of canonical Notch signaling in aBM myeloipoiesis, in steady-state and following transplantation.

**Methods:** BS-SLJDC45.1, Rbpj deficient mice were used. Flow cytometry (FACS) was applied for phenotypic analyses. Gene expression levels were measured by real-time reverse transcription PCR. MK, E and GM in vitro colony-forming potentials were applied in mouse colony assays. For transplantation studies, lethally irradiated recipients were competitively transplanted (1:1) and reconstitution assessed 7-9 weeks after transplantation.

**Results:** FACS staging of GM, Mk and E progenitors in aBM of old flanked Rbpj mice crossed to both 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, E and GM in vitro colony-forming potentials were applied in mouse colony assays. For transplantation studies, lethally irradiated recipients were competitively transplanted (1:1) and reconstitution assessed 7-9 weeks after transplantation.

**E1110 DEVELOPMENT OF A 3-DIMENSIONAL CULTURE TO MIMICK THE BONE MARROW MICROENVIRONMENT AND RECAPITULATE DRUG RESISTANCE FOR IN VITRO STUDY**

**Background:** Chronic myeloid leukemia (CML) is a haematological malignancy caused by the acquisition of the BCR-ABL1 oncogene. Demonstration of the central role of BCR-ABL1 kinase activity in CML pathogenesis led to the development of 1-specific tyrosine kinase inhibitors (TKI). Most patients on imatinib attain good clinical and molecular responses, despite the persistent presence of a low level of therapy-refractory leukaemia stem cells (LSCs), which reside in the bone marrow niche. However, in a significant majority of patients these cells eventually provide a reservoir for disease relapse and subsequent malignant progression. A greater understanding of the biology of imatinib-resistant 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.

**Methods:** Development of a 3-dimensional culture using fibre scaffolds to mimic bone marrow microenvironment in order to study the mechanism of resistance to anti-leukaemia agents.

**Results:** PMMA-based 3D scaffold and compared the growth of CML and AML cell lines grown in this scaffold in the presence 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.
fibres. Treatment of K562 or HL60 cells with imatinib or doxorubicin respectively resulted in a lower level of apoptosis in cells grown on the 3D scaffold compared to those grown in 2D culture. Further development of this 3D culture by adding stromal HS-5 to the scaffold reduced even further the sensitivity of K562 or HL60 to imatinib or doxorubicin, respectively.

Figure 1.

Summary/Conclusions: The relative resistance to either imatinib or doxorubicin that we observed in cells grown in 3D culture supports a role for the bone marrow matrix in the protection of leukaemic cells against chemotherapy 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 leukaemia and for the discovery of new agents capable of eradicating quiescent leukaemic stem cells.

E1113

WHOLE EXOME SEQUENCING REVEALED SEQUENTIAL GAIN OF MUTATIONS IN TWO CASES OF DONOR CELL HAEMATOPOETIC TRANSPLANTATION MALIGNANCY AFTER HEMATOPOETIC TRANSPLANTATION J. Suárez González1,2, C. Martínez-Laperche2,3, M. Kwon2,3, G. Rodríguez-Macias3, A. Figuera4, A. Balas5, N. Martínez6, P. Balsalobre2,3, D. Serrano2,3, M.Á. Piris6,7, J.L. Vicario5, J. Gayoso2,3, J.L. Díez2,3, I. Buño1,2,3

Background: Santander, Spain

Methods: Case 1: A 43-year-old female diagnosed with lymphoblastic leukaemia- B (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 acute myeloid leukemia from his HLA-identical brother. WES (SureSelect-XT Human-exon 50Mb) was performed by next generation sequencing (HiSeq) on donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT. The exome of donor SCs and BM samples, from case 1, were aligned to the human reference genome (GRCh 37/hg19) and donor SCs and 9 BM samples were aligned to GRCh 38/hg38 in the second case.

Results: WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukaemia in bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukaemogenesis. (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 leukaemogenesis in these cases (Figure 2).

Summary/Conclusions: The present study reveals a process of sequential clinical (transitions), promoted by the acquisition of additional distinct 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 to allo-HSCT. Although the cause of donor cell derived haematological malignancy onset seems to be multifactorial, the infussion of a SCU with pre-leukemic potential in a context of 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 B. Depreter1,2,*, K. Vandepoele3, B. De Moerloose4, B. Denys3, J. Philippé1,3, T. Lammens2

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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 leukaemic cell populations organized in a hierarchical fashion, and leukaemic stem cells (LSCs) residing at the apex herein. Unfortunately, gene expression profiling is commonly performed on unfractinated 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' tumour 1 (WT1)) and reference gene (RPL13a, GAPD) expression. Expression analysis was performed in unfractinated bulk leukemic cells as well as blasts and rare subsets of leukemic cells, e.g. LSCs. FCM analyses were performed on a FACSCount II (BD Biosciences) with set-up according to EuroFlow guidelines. Infinicyt™ (Cyognos®) was used for data analysis and mean fluorescence intensities (MFI) values (with/without normalisation) were interpreted. P-values < 0.05 were considered significant.

Results: mRNA expression quantified by PrimeFlow™ significantly correlated with data obtained by RT-qPCR and remained detectable in rare (0.1%) cell populations. WT1 expression was shown to be statistically significantly higher in bulk leukemic cells of those patients characterized by WT1 overexpression, as defined by RT-qPCR, showing a mean 52% MFI upregulation by PrimeFlow™ if WT1 was overexpressed as present, as shown 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, compared to patients with normal WT1 expression levels, although only statistically significant in 11/19 cases. The expression of WT1 was statistically significantly higher in CD34+CD38+ cells compared to LSCs.

Figure 1.

Methods: Case 1: Patient 1: 5 BM samples were aligned to GRCh 37/hg19 in the first case and 9 BM samples were aligned to GRCh 38/hg38 in the second case. Case 2: WES (SureSelect-XT Human-exon 50Mb) was performed by next generation sequencing (HiSeq) on donor stem cells (SCs) infused as well as on BM samples from recipient after allo-HSCT. The exome of donor SCs and 5 BM samples, from case 1, were aligned to the human reference genome (GRCh 37/hg19) and donor SCs and 9 BM samples were aligned to GRCh 38/hg38 in the second case.

Results: WES analysis revealed progressive emergence of multiple somatic mutations probably related to the development of leukaemia in bone marrow samples post allo-HSCT (Figure 1). Both SCs showed alterations that may be involved in leukaemogenesis. (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 leukaemogenesis in these cases (Figure 2).

Summary/Conclusions: The present study reveals a process of sequential clinical (transitions), promoted by the acquisition of additional distinct 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 to allo-HSCT. Although the cause of donor cell derived haematological malignancy onset seems to be multifactorial, the infussion of a SCU with pre-leukemic potential in a context of 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.
Summary/Conclusions: Key mRNA target expressions in AML, e.g. WT1 gene expression, could be evaluated using PrimeFlow™ RNA assay, including rare and heterogeneous cell populations herein, e.g. LSCs. This study demonstrates that PrimeFlow™ is a technique of interest for the discovery of novel LSC-specific targets.

E1115
POTENTIAL PREDISPOSING GERMLINE MUTATIONS IN PATIENTS WITH CONCOMITANT MYELOID AND LYMPHOID MALIGNANCIES
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Background: Recent findings have suggested that mutations predisposing the development of either acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL) may arise in pre-leukemic hematological stem cells. In addition, genes involved in epigenetic regulation, such as TET2, and DNA processing, such as SFB3, 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.

Methods: We aimed to identify mutations in patients with concomitant AML and diagnosis of de novo AML or myelodysplastic syndrome (MDS) and in patients with concomitant therapy-related AML (t-AML) and CLL.

Results: Three patients with de novo AML and CLL, one patient with MDS and CLL, one patient with chronic myelomonocytic leukemia (CMML) and CLL, and two patients with t-AML and CLL. The patients’ diagnoses were based on the evaluation of the morphology, immunohistochemistry, cytogenetics, and flow cytometry analysis in accordance with the WHO classification.

Aims: In this study, we aimed to investigate a possible common genetic origin of hematological cancers in patients with concomitant CML and de novo AML or myelodysplastic syndrome (MDS) and in patients with concomitant therapy-related AML (t-AML) and CLL.

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 DNA processing, such as SFB3, 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 CML and de novo AML or myelodysplastic syndrome (MDS) and in patients with concomitant therapy-related AML (t-AML) and CLL.

Methods: The presence of concomitant lymphoid and myeloid malignancies in patients is rare, however we managed to include 3 patients with de novo AML and CLL, one patient with MDS and CLL, one patient with chronic myelomonocytic leukemia(CMML) and CLL, and two patients with t-AML and CLL. The patients’ diagnoses were based on the evaluation of the morphology, immunohistochemistry, cytogenetics, and flow cytometry analysis in accordance with the WHO classification. For each patient mononuclear cells (MNCs) from blood or bone marrow were isolated using Ficoll gradient centrifugation and used for fluorescence activated cell sorting (FACS) of the malignant clones and the T-cells. Paired 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) mapping cell software. Variants with a frequency of 5% or above were called.

Results: Identified possible pre-existing 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 in the germline and/or 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), SMARCA1 (2114C>T, T705I), HELQ (393_397delAGGTG, D132fs*16), SWI5 (652C>T, R218*), LIG1(2168A>G, Q761R) and PRKDC(802G>A, G301Y). 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. De novo AML and CLL, we identified a potential damaging germline variant in an epigenetic regulator believed to play a role in normal and malignant hematopoiesis.

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.

Results: When DNMT3A mutations in CHIP were compared to mutations in AML the R882 residue was found to be the most frequently mutated residue in both AML and CHIP. Figure 1 clearly illustrates the mutations in comparison to AML. However, only 13% of all reported mutations were found at the R882 residue in CHIP, while in AML 60% DNMT3A mutations are found at the R882H mutations.

Summary/Conclusions: Analysis of the mutational landscape of CHIP has clearly highlighted the role of DNMT3A mutations in clonal 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.
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.

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 (rrt) 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 rtt 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 rtt 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 rrt disease; the median number of prior treatment lines was 5 (2–11) and 21 patients received prior brentuximab vedotin. To investigate the association of baseline blood count parameters (white blood cell count (WBC), relative monocyte count (RMC), relative neutrophil count (RNC), relative lymphocyte count (RLC) and relative eosinophil count (REC)) with outcome after PD1 inhibition, we used the last differential blood count performed immediately prior to the first received dose of nivolumab.

Results: RMC, RNC and RLC did not have a prognostic impact on PFS, whereas higher WBC ≥ 7.78x10³/µl and lower REC<1.7% were associated with worse PFS in both univariate and multivariate analysis. We constructed a simple score to prognosticate PFS. By adding 1 point each for WBC ≥ 7.78x10³/µl and REC<1.7% to the score, we could clearly differentiate a low (score=0), intermediate (score=1) and high risk (score=2) group for disease progression (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.

Results: ROC curves and sequential cut-offs (1.8–3.5 by 0.1 increments) were used to explore the potential impact of b2m on PFS and OS.

Methods: We analyzed 864 patients with HL treated with ABVD/C T/C early stage patients, with 10-year FFP of 99% (p=0.001). In advanced stages, b2m emerged as an independent prognostic factor for FFP post-adjustment for IPS factors, ESR and B-symptoms at both 2.1mg/L (HR 2.00, p=0.01) and 2.4mg/L (HR 2.14, p=0.01). In a 2.4mg/L cut-off, the 10-year FFP was 81% vs 71% (p=0.003). However, the best cut-off was the observed median value of this series, calculated at 2.1mg/L, with 10-year FFP of 84% vs 71% (p=0.0001). In early stages (IA/IIA) significant results were obtained at cut-offs between 1.8 and 2.1mg/L. The best cut-off was 1.9mg/L, a close approximation of the median b2m level of early stage patients, with 10-year FFP of 99% vs 78% (p=0.003). In advanced stages, none of the cut-offs yielded statistically significant results (borderline at 2.0mg/L, 10-year FFP 77% vs 67%, p=0.057). Multivariate Analysis: B2m levels remained significant for FFP after adjustment for IPS factors, ESR and B-symptoms at both 2.1mg/L and 2.4mg/L cut-offs (hazard ratio (HR) 1.78, p=0.001 and 1.41, p=0.04 respectively). ROC curves and sequential cut-offs (1.8–3.5 by 0.1 increments) in the whole series of 864 patients were used to explore the potential impact of b2m on FFP and OS.

Results: We analyzed 864 patients with HL treated with ABVD+C T/C (1990–2016) and selected solely based on the availability of pretreatment b2m levels. B2m [P1] levels (upper normal limit 2.4mg/L) were analyzed according to other baseline features and prognostic factors as well as according to the outcome. Freedom From Progression (FFP) was defined as time between treatment initiation and treatment failure (primary refractoriness, PR with switch to alternative CT or relapse); deaths of unrelated causes were censored. Overall Survival (OS) was measured from treatment initiation to death of any cause. ROC curves and sequential cut-offs (1.8–3.5 by 0.1 increments) were used to explore the potential impact of b2m on FFP and OS.

Results: The median follow-up for currently living patients was 88 months. Univariate Analysis: FFP was significantly inferior in patients with higher b2m at all tested cut-off points. At 2.4mg/L (normal versus elevated) the 10-year FFP was 81% vs 71% (p=0.003). However, the best cut-off was the observed median value of this series, calculated at 2.1mg/L, with 10-year FFP of 84% vs 71% (p=0.0001). In early stages (IA/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 99% vs 78% (p=0.003). In advanced stages, none of the cut-offs yielded statistically significant results (borderline at 2.0mg/L, 10-year FFP 77% vs 67%, p=0.057). Multivariate Analysis: B2m levels remained significant for FFP after adjustment for IPS factors, ESR and B-symptoms at both 2.1mg/L and 2.4mg/L cut-offs (hazard ratio (HR) 1.78, p=0.001 and 1.41, p=0.04 respectively). ROC curves and sequential cut-offs (1.8–3.5 by 0.1 increments) in the whole series of 864 patients were used to explore the potential impact of b2m on FFP and OS.

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The objective of the present study was to evaluate the significance of PET 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 (65%) patients had advanced disease and mean international prognostic score (IPS) was 3.5±1.4. Fifty nine (63%) patients received 1st 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 59% 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 PET and EOT-PET. 50 patients had NED on PET, 20 had PR, 1 SD and 1 PD. NED EOT-PET was achieved in 47/50 (94%) patients who had NED PET, 12/20 (60%) patients who had PR PET and none of the patients with SD/PD PET (p<0.01). In patients with either NED or PR on PET, relapse occurred in 11 (15%) patients and 5 year PFS and OS were 62% and 85%, respectively. The 5 year PFS of these patients differed according to the depth of response on PET - 69% vs 45%, (p=0.02, fig. 1) in patients achieving NED vs PR, while 5 year OS did not reach statistical significance, 90% vs 71% (p=0.08). Restricted analysis, evaluating only 59 patients who were treated with ABVD, showed similar results with 84% of NED PET vs 45% of PR PET achieving NED on EOT-PET (p<0.01). Outcome differed according to the depth of response in PET 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).

Summary/Conclusions: We present a cohort of elderly patients with HL, most were treated with ABVD. Outcome was comparable or even superior to previously published cohorts. Traditional outcome measures for HL have not been extensively validated in the elderly. PET and EOT-PET, known to be highly predictive for PFS in young HL patients, appeared to be highly predictive in elderly individuals. The improved prognosis, suggested by our results, may be related to the high rate of PET which was used in this cohort. The importance of this tool in HL in the elderly is emphasized by the diminished prediction power of the traditional outcome measures in elderly HL patients.

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.

Figure 1.

Summary/Conclusions: High-dose bendamustine plus brentuximab combination is effective and has a favourable toxicity profile in the treatment of refractory and relapsed Hodgkin lymphoma.

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 events occurred were recorded and classified for type and grade using NCI-CTCAE criteria (v.4.0).

Results: In arm A, the overall response rate (ORR) was 40% (4/10 patients), with 4 (40%) complete remission (CR) and 6 (60%) progressive disease (PD). Hematological toxicity was grade 3 thrombocytopenia in 4 patients (40%) and bone marrow aplasia in 1 patient (10%); extra-hematological toxicity was gastrointestinal toxicity of grade 2 in 6 patients (60%) and grade 1 in 3 patients (30%). In arm B, ORR was 60% (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 2 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.
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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, patients treated with EBV or cytotoxic late toxicities, such as, gonadal toxicity that can result in permanent sterility.

Aims: to evaluate different aspects of fertility (menstrual status, pregnancy, and menopause) in women with HL and NHL in reproductive age before and after chemotherapy.

Methods: By 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) 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 [ES1] (64%) of the patients had a stage I-II. All HL patients were treated with ABVD, whereas the NHL patients were treated with R-CHOP (20%) or similar regimens (16%), respectively. Radiotherapy was delivered to the 62/109 (57%) of the sample. Complete Remission (CR) was obtained by the 101/109 (93%) and only 16/101 (16%) relapsed. Considering the gynecologic history of the patients there were no statistically significance difference between the regularity of menses and the event of an abortion pre and post treatment. As for pregnancies, 35% of patients had children before therapy and 17% after. Among these 109 patients, 68/109 (62%) received gonadotropin-releasing hormone (GnRH) analogues and/or oral contraception, while 41 (38%) were not treated with hormonal therapy. Among the 68 patients who received hormonal therapy regular menses recovered in 51/68 (76%) in 6 months and in those of the control group a recover of menses was observed in 20/41 (48%). This difference was statistically significant (P<0.05). The same was observed as for early menopause. In this case excluding patients who had a natural menopause, a lower cases of early menopause was observed in those who received hormone therapy (6/85, 7% vs 11/20, 55%); P<0.05. Considering only the 81/109 (74%) patients who had regular menses after chemotherapy, 61/81 (75%) received hormonal therapy and 20/81 (25%) were not treated with hormonal therapy. Before treatment for lymphoma, 16% of patients belonging to the hormonal group had pregnancies versus 45% of the control group (P<0.05). Following therapy, pregnancies were observed in 23% of those receiving hormonal therapy vs 5% of the control group (P<0.05).

Summary/Conclusions: The use of hormonal therapy is fundamental not only to favor of pregnancies and motherhood but in particular to avoid the consequences of an irregular cycle or an early menopause with its symptoms and clinical implications.

E1124

25(OH) VITAMIN D SERUM LEVELS IN HODGKIN LYMPHOMA

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Background: 25(OH)VITAMIN D SERUM LEVELS IN HODGKIN LYMPHOMA

E1125

NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA: A NEW RISK ADAPTED TREATMENT STRATEGY BASED ON RITUXIMAB

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Background: Nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) is a rare variant of Hodgkin’s lymphoma (HL), that only accounts for 5% of all HL. Due to its rarity, consolidated and widely accepted guidelines of treatment still lack for this type of HL. Due to NLPHL cells expression of CD20, targeted therapy with Rituximab (R), a chimeric anti-CD20 monoclonal antibody, has been explored as a treatment option.

Aims: This study analyzed two different risk-adapted therapeutic strategies to cure patients newly diagnosed with NLPHL. The aim was to compare the efficacy and safety of the conventional chemotherapy plus irradiation versus the R-including treatment of patients with NLPHL.

Methods: Within a retrospective study, we collected the medical records of 24 consecutive adult patients with NLPHL, taken from the total of 484 patients with HL, who referred to our institution from 1 October 2001 to 31 July 2014. According to our institutional guidelines, the 12 patients diagnosed from October 2001 to November 2007 received a treatment based on ABVD with/without involved-field radiotherapy (IFRT). Treatment was modulated according to the stage. The 9 patients with stages I and II received 4 courses of ABVD plus IFRT, while 3 patients in stages III or IV received 6 cycles of ABVD. The subsequent 12 patients (diagnosis from December 2007 to July 2014) received R (375mg/m²) alone or combined with ABVD. The stage-adapted strategy of therapy was applied for these patients, as well. The 5 patients with early favourable disease according to the stage and baseline EORTC risk factors, received R as single agent (once per week for four consecutive weeks) followed by R maintenance (MR) (once every three months for 2 years); the 2 patients with early unfavorable stage were treated with R (once per month on day 1) plus 4 cycles of ABVD, while the remaining 5 advanced stage patients received R (on day 1 and 15) plus ABVD for 6 weeks. The primary end-point was DFS rate, and secondary end-points were ORR and treatment-related toxicity evaluation.

Results: At final restaging, 4 weeks after the cycle of treatment or completion of IFRT, 23/24 patients (95.8%) were in CR while one patient showed refractory disease and was addressed to rescue therapy with autologous hematopoietic stem cell transplantation. There were no statistically significant differences in OS between the 2 groups. However, DFS was significantly higher in the R-adapted arm than in the conventional arm (P=0.021).

Summary/Conclusions: The R-adapted strategy resulted in a significantly better DFS rate compared to the conventional therapy. Further investigation is needed to confirm these results in a larger study population.
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. Radio-conventional radio-chemotherapy either in term of ORR and of DFS while sparing long term toxicity usually seen in patients affected by classical HL who receive chemo and irradiation.

E1126

CASE-BASED LEARNING IN CONTINUING EDUCATION: IMPROVING HEMATOLOGIST/ONCOLOGIST EVIDENCE-BASED DECISIONS FOR PREVENTING HODGKIN LYMPHOMA POST-TRANSPLANT RELAPSE

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Background: Several prognostic factors have been identified as associated with a higher rate of relapse after autologous stem cell transplantation (ASCT) for patients with Hodgkin lymphoma (HL). Due to the rarity of this disease, many hematologists/oncologists (hem/oncs), especially those in the community setting, lack experience in correctly identifying patients who may be at risk of post-transplant relapse. Proper risk assessment and understanding of treatment options in the pre- and post-transplant setting are critical to ensure optimal long-term 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 4-6 questions that “tested” learner knowledge and competence before delivering the education focused on the optimal approach to the case using evidence-based medicine. Case questions assessed degree of patient risk for disease relapse or progression prior to ASCT and consolidation strategies, taking into consideration patients’ prior received therapies. To assess educational effectiveness, participants served as their own controls by responding to a series of same questions again after (post-assessment) exposure to the 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<0.001); 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<0.001); 5% relative improvement in knowledge regarding the efficacy of brentuximab vedotin in relapsed/refractory HL after ASCT (P<0.001); Responses to a self-efficacy question indicated that 42% of hematologists became more confident in managing 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.

E1127

QUANTITATIVE PET PARAMETERS PREDICTS OUTCOME IN PATIENTS WITH HODGKIN’S LYMPHOMA

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Background: Positron emission tomography [18F] fluoroodeoxyglucose (FDG-PET) has emerged as the standard response assessment after 1st line therapy for classical Hodgkin’s lymphoma (HL). Quantitative PET parameters are not well established as a predictive factor for disease progression in HL.

Aims: Thus, the aim of this study was to test the hypothesis that tumor burden characterized by mean standardized uptake value (SUVmean), maximum SUV (SUVmax), metabolic tumor volume (MTV) and total lesion glycosylation (TLG) could be independent prognostic factors.

Methods: We analyzed the relation of absolute value PET parameters, negative predictive value (negative PET scan and no treatment failure, NPV) and positive predictive value (positive PET scan and treatment failure, PPV) with event-free survival (EFS) or overall survival (OS). Quantitative PET parameters of the baseline (PET-1), interim (PET-2) and end of treatment (PET-3) PET-CT scans were investigated in the retrospective study. MTV was computed by using the 41% maximum SUV thresholding method, and the optimal cut-off for survival prediction was determined.

Results: Thirty one patients with HL and a stage I-II—51.6%, III-IV—48.4% consecutively admitted from April 2009 to December 2016, by 5 Ukrainian hematological centers were included in the analysis. Patients were staged at baseline, after 2-4 cycles of chemotherapy with PET/CT and at the end of chemotherapy. All patients were treated with ABVD, BEACOPP-14/sec. All 31 patients achieved CR or PR and 67.7% had a negative PET-2, while 16.3% had a positive PET-2. Patients with negative PET-2 and positive PET-2 had CR rates of 64.5% and 12.1%, respectively, which yielded a PPV of 26% and NPV of 74%. ROC analysis revealed that PETV and NPV are an important markers associated with EFS in patients with HL (Se=100%; Sp=100%; AUC=1.0). 3-year EFS was 100% for NPV patients and 12% for PPV patients, which was statistically significant (p<0.001). At the last cycle of chemotherapy, OS was 100% for NPV patients and 12% for PPV patients, respectively (p<0.01). Quantitative parameters at PET-1 and PET-2 were not statistically significant in predicting clinical outcome in this study. This may be due to the small sample size in our study. PET-3 was negative in 67.7% cases. ROC analysis showed that 3MTV at PET-3 is an important marker associated with reduced EFS in patients with HL (Se=75%; Sp=100%; AUC=0.97, p<0.001). 3-year EFS was 80% and 25% in patients with ∑MTV <4.75 and ∑MTV >4.75, respectively (p=0.005). Also, ROC analysis revealed that TLG at PET-3 was associated with decrease EFS in patients with HL (Se=75%; Sp=100%; AUC=0.97, p<0.001). Multivariate analysis confirmed ∑MTV and TLG at PET-3 were the only significant variables for EFS with HRs of 1.07 [95% confidence interval(CI) 1.0–1.15, p=0.003] and 2.9 [95% (CI) 9.13–0.05, p=0.05], respectively.

The PET-3 SUVmax and SUVmean were not statistically significant in predicting EFS.

Summary/Conclusions: Quantitative PET parameters may play a predictive role for identifying patients at high risk of treatment failure. These results should be evaluated prospectively in larger cohorts with longer follow-up.
Indolent Non-Hodgkin lymphoma - Clinical

E1128

Abstract withdrawn.

E1129

BIOMARKER ANALYSIS OF PATIENTS WITH FOLLICULAR LYMPHOMA TREATED WITH IBRUTINIB IN THE PHASE 2 DAWN STUDY


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Background: Ibrutinib, a first-in-class, oral, covalent inhibitor of Bruton’s tyrosine kinase, has demonstrated robust clinical activity and is approved in various B-cell non-Hodgkin’s lymphomas. To assess the efficacy and safety of ibrutinib in patients (pts) with follicular lymphoma (FL), the DAWN study (FLR2002, NCT01779791) investigated single-agent ibrutinib in chemotherapy-naïve patients (pts) with follicular lymphoma (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 International Working Group (IWG) criteria. Secondary endpoints include 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.

Methods: DAWN study was an open-label, multicenter, single-arm, phase 2 study of ibrutinib in pts with CIT-refractory (i.e., ≥3 prior lines of therapy and progressive disease [PD]) ≤12 months after last dose of a CIT regimen. All pts received ibrutinib (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 peripheral blood mononuclear cells (PBMCs) 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− at C3D1 in 14 responders (CR + PR, median decrease ≥17.2% of CD4+CD25+CD127− in 43 non responders). Th1 cytokines interferon-gamma (IFN-γ) and IL-12 were increased in responders but decreased in nonresponders (p=0.0025 and p=0.035, respectively). 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 responders pts at early time points, downregulation of T-regulatory cells 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 Treg were not decreased. 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 immun-ontcology therapies may prove beneficial.

E1130

DYNAMO: THE CLINICAL ACTIVITY OF DUVELISIB IN PATIENTS WITH DOUBLE-REFRACTORY SMALL LYMPHOCYTIC LYMPHOMA IN A PHASE 2 STUDY


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Background: Duvelisib is an oral, dual inhibitor of PI3K-δ,γ in development for the treatment of hematologic malignancies. DYNAMO is a Phase 2 study to evaluate the safety and efficacy of duvelisib in a double refractory iNHL population, which included 28 patients (pts) with small lymphocytic lymphoma (SLL). This study aimed to establish the safety and tolerability of duvelisib.

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.

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 (25%) or high (25%) performance status. Median time from last antitumor 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 PFRs. Four (14%) pts had a best response of SD and 3 (11%) pts had a best response of PD. 2 pts were unevaluable for response. Per Investigator assessment, the ORR was 79% (including 1 CR). Median time to IRC response was 1.9 months (range 1.4-5.5). 93% of pts had a reduction in nodal target
lesions. Among the 19 SLL pts with a response per IRC, the median DOR was 9.8 months. The median PFS among all SLL pts was 11.3 months, while the median OS was not reached. The estimated probability of survival at 12 months was 83.9%. Among all pts treated (n=129), AEs were mostly Gr 1-2. Most common ≥ Gr 3 AEs were transient cytopenias (neutropenia [23%], anemia [12%], and thrombocytopenia [10%]), and diarrhea (15%). 4 SLL pts had SAEs that led to discontinuation of duvelisib: NSCLC, neuroendocrine carcinoma of the skin, pseudomembranous colitis, and pneumonia. Two SLL pts has a fatal AE, 1 pneumonia and 1 viral infection.

Summary/Conclusions: In DYNAMO, duvelisib showed clinical activity in a double-refractory SLL population (68% ORR, median DOR 9.8 mo., 93% with a reduction in target lesions). Duvelisib was generally well tolerated, with a manageable safety profile with appropriate risk mitigation. Duvelisib monotherapy appears to have a favorable benefit-risk profile in double refractory SLL. Updated clinical data will be available at the time of presentation.

E1131
Abstract withdrawn.

E1132
WALDENSTROM MACROGLOBULINEMIA: UK REAL WORLD EXPERIENCE
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Background: There are few randomised controlled trials in Waldenström macroglobulinemia (WM) due to its rarity and indolent nature. As a result, there is no standard treatment approach and management is variable. Aims: The aim of this retrospective study was to review “real world” management of WM in the UK and correlate this with survival outcomes.

Methods: All patients with a diagnosis of WM seen at ULCH between 01/07/2002 and 31/12/2016 were included. Patient characteristics, presenting features, lines of treatment, responses and overall outcome were recorded. Response to the ongoing antiviral therapy was achieved in 77% of cases. Median progression-free survival in patients with IL + C treated with antiviral treatment was 42 months, in patients with IL without viral hepatitis markers.

Methods: The study included 107 patients with indolent lymphoma who were identified in the blood markers of hepatitis C. Results: Histological types were follicular lymphoma - 74%, marginal zone lymphoma - 32%. The age of patients ranged from 28 to 82 years (median 50). Men / women ratio was 1: 1. Stage I + II were in 3%, III stage was in 24% of patients. Primary extranodal lymphoma was diagnosed in 33% of patients. Extranodal lesions: splenic lesion - in 53% of patients, liver injury - 21% of the patients, the bone marrow - 62% of patients. LDH > 450 IU / l was at 76% of cases, ALT > 40 IU / l was at 82% of cases, albumin < 35 g / l was at 31% of patients. 57 patients were treated with interferon and Ribavirin as a first-line treatment. Treatment lasted for 2 years after reaching the antitumor effect. 50 patients were treated with immunochemo therapy (R-CHOP, R-CVP) as a first-line treatment. Antiviral therapy was effective in 88% patients, immunochemo therapy was effective in 64% of patients. Median progression-free survival in patients with IL + C treated with antiviral treatment was 42 months. In patients with IL + C treated with immunochemo therapy - 19 months (p=0.00001). Five-year overall survival was 67% and 32%, respectively (p=0.0003). It was diagnosed disease relapses after immunochemo therapy in 39 patients. All the patients in the second-line was received antiviral treatment. The size of the disease was ongoing in 73% of cases. Medi an progression-free survival in relapsed lymphoma was 31 months.

Summary/Conclusions: Antiviral therapy in first-line and relapse of disease surpasses all the indicators of efficiency of treatment IL + HCV. In this category of patients preferred option is to conduct anti-viral treatment.
(R every 8 weeks for 4 or 12 doses) still appears as an optional part of the therapy (NCCN V3:2016). Radiomunotherapy with 90Ytrium-ibritumomab tiuxetan (90Y-IT) is available in our institution since 2006 and more than 100 patients have been treated with RIT since then. Here an institutional analysis focus in their use as consolidation is presented

Aims: To analyze the experience with 90Y-IT as a consolidation therapy in patients in CR after first-line therapy.

Methods: A retrospective analysis was performed including all the patients that have received RIT with 90Y-IT. Inclusion criteria were: patients 18 years or older with a grade 1-2a follicular lymphoma, RIT was received as a consolidation therapy in complete response (CR) after a first-line therapy. Demographic and follow-up data were included. International working group (IWG) criteria of response was used. Progression free survival (PFS) was calculated from the date of RIT to the date of a confirmed relapse according IWG criteria, overall survival (OS) was calculated from the FL diagnosis to the last contact.

Results: A total of 31 FL patients have received 90Y-IT been in CR after a first-line therapy and were included for the study. Mean age at diagnosis was 61.2 (29-86) years with a female predominance (19, 61.3% vs 12, 38.7%). 80.6% (26) with ECOG 0-1 and 19.4 ECOG 2. A third of them (10, 32.3%) were treated for stage III-IV. As long-term events one 82 years old patient developed a colon infiltration demonstrated by flow cytometer or biopsy. There were no patients with bulky disease. Stages: I: 7 (22.6%), II: 2 (6.7%), III: 9 (29.1%), IV: 12 (38.7%). As first-line therapy the patients received: Rx4: 11 (35.5%) cases, R-Chlorophosphamide vinristine prednisone (COPx4): 3 (9.7%) cases and 17 (54.8) R-Chlorophosphamide doxorubicin, vinristine and prednisone (R-CHOPx4). The median follow-up was 58.0 (10-107) months. During this time only 5 (16.1%) of patients have relapsed and need another therapy. None of the patients that have received R-CHOP+90Y-IT have relapsed; the relapsed patients received Rx4 (4) and R-COP (1). The median PFS after 90Y-IT has not reached, the mean was 83.3 (71.7-94.9) months, see Fig 1. Four (12.9%) patients have died, none of them were relapsed and the mortality was due other causes. The median OS was not reached, the mean was 95.8 (85.6-106.1) months. As long-term events one 82 years old patient developed a colon cancer after 67 months of RIT; one 72 years old female a breast cancer after 17 months of RIT and one 71 years patient amgUS after 24 months of RIT, none of them related with mortality events.

Summary/Conclusions: The use of immunotherapy with rituximab or combined schedules with immunochemotherapy (R-COP and R-CHOP) followed by consolidation with 90Y-IT remains as a valid option for follicular lymphoma patients. After ~6 years of follow-up: 63.8% (Rx4+RIT), 66.7% (R-COP+RIT) and 100% (R-CHOP+RIT) of patients continue with complete response and off of therapy.

E1135

ASSESSING RISK OVER TIME IN PATIENTS WITH SYMPTOMATIC WALDENSTROM MACROGLOBULINEMIA (WM). A STUDY ON 114 PATIENTS


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Background: By contrast, with follicular lymphoma (J Clin Oncol 2015;33:2616) or other chronic hematological malignancies (Blood 2009;114:1299; Blood 2016;128:902), few studies attempted to decipher the evolution of pts with WM, a disorder associated with delayed response to therapy in some pts.

Aims: To assess the prognostic role during the clinical course of initial interna tional prognostic index (IPSSWM), response and progression (according to 6th International Workshop guidelines) on survival.

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 after 1st line therapy was 70%. Sixty-two, 37 and 19 pts received a 2nd a 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 EJD 2016.

Results: Median survival after 1st line was estimated 79 months. It was esti mated 69 and 65 months after 2nd line and 3rd line respectively. High IPSSWM (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 (Grimbsch and Theerneau test: p=0.053). Consequently, Dxy concordance index obtained in multiple landmark 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 >25%) and progression had significant prognostic value. By contrast, onset of progression and initiation of 2nd line therapy, retained prognostic values for SAFTI (p=0.0038 and p=0.004 respectively). Only 2 thresholds in SMIC defined a response status (observed between onset of response and progression) of prognostic value for SAFTI: namely >25% reduction in SMIC (i.e. minor response or better: p=0.041) and >50% (i.e. partial response or better: p=0.026). In similar Cox models with hiPSSW M, onset of progression (p=0.0034) and 2nd treatment initiation (p=0.0031) retained independent prognostic value inside hiPSSWM (p<0.0026). Times elapsed from the initiation of 1st line therapy to 1st progression and to the ini tiation of 2nd line therapy had no prognostic value for subsequent survival. In similar Cox model of survival after 2nd line therapy with time dependent covar iate 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 hiPSSWM decreased in part during the first 6 years of evolution. Onset of progression and 2nd treat ment initiation provided additional prognostic information for predicting SAFTI. Therefore progression-free survival or time to next treatment may be satisfac tory surrogate endpoint of SAFTI in WM. Further international collaborative studies are mandatory for this purpose. Assessing response in more advanced phase of the disease may require specific tools.

E1136

TIME TO NEXT TREATMENT ANALYSIS FOR EARLY AND ADVANCED STAGES OF MYCOSIS FUNGOIDES /SEZARY SYNDROME TREATED WITH BEXAROTENE AND PUVA IN COMBINATION

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Background: Bexarotene is a synthetic retinoid effective in early and advanced stages of Mycosis Fungioides (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: We aimed to evaluate TTNT together with the usual time-to-event measures (OS and EFS) in our series of 21 refractory and/or relapsed patients with MF treated with Bexarotene and PUVA combination. We recently 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 designed “mini” 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.
Results: We enrolled 21 patients, 12 males and 9 females, with median age of 67 years (range, 30-77), of which 15 affected by early MF (13 with stage IB, 2 with stage IIA) and 7 by advanced disease (2 with stage IIB, 2 with stage IIIA, 1 with stage IIIB and 1 with stage IVA). Six patients had previously received PUVA therapy only, while fifteen patients had received other therapies. The protocol proved to be effective, well tolerated and able to induce an overall response of 55.6% at the end of induction phase (93.4% of early stage patients and 66.6% of advanced stage patients) and of 76.2% at the end of maintenance phase (86.7% of early stage patients and 14.2% of advanced stage patients). Median follow up for all patients was 85 months (6-118) with respectively 98 months (21-118) for early stages and 46 months (6-102) for advanced stages. For the entire cohort, median OS, PFS and TTNT were not reached; mean values of OS, PFS and TTNT were respectively, 105, 103 and 79 months, and median EFS was 58 months. For advanced stage patients, median OS, PFS, EFS and TTNT were 32, 29, 18 and 39 months respectively.

Summary/Conclusions: Our combination treatment seems to have superior TTNT compared to data published in the literature for PUVA and bexarotene used in monotherapy. When considering early and advanced MF, 66% of our patients are estimated to be free from further treatment at 2 years, a higher percentage compared to the results of Hughes et al. (Blood, 2015) for patients treated with PUVA (54.2%) or bexarotene (36.8%) as single agents. Moreover, TTNT seems to be longer in our study than in the study by Hanel et al (AJH 2016) on patients treated by retinoids in monotherapy, respectively 79 vs 60 months (mean TTNT values) in the early stages and 39 vs 9 months (median TTNT values) in the advanced stages. We believe that our results strongly suggest a synergistic or additive effect between PUVA and bexarotene compared to either agent alone in the treatment of both limited-stage and advanced stage MF.

E1137
PERIPHERAL BLOOD INVOLVEMENT IN PATIENTS WITH ADVANCED STAGE FOLLICULAR LYMPHOMA: CLINICAL-BIOLOGICAL CHARACTERISTICS AND PROGNOSTIC IMPACT
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To describe the clinico-biological characteristics and to determine the clinical-biological characteristics and to determine the prognostic impact of blood involvement in patients with advanced FL.

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 involvement (PB+) defined by the presence of circulating FL cells by morphology, further confirmed by immunophenotyping. The main clinical and biological characteristics, response to treatment and outcome were analyzed.

Results: Patients with PB+ more frequently had splenic involvement, anemia, elevated β2-microglobulin and LDH and high FLIPI score than those without PB involvement (PB-) and differences were statistically significant. There were no differences concerning the proportion of patients undergoing a watchful waiting approach (7% vs 9%), type of treatment, or overall response rate (93% vs 88%) and complete response rate. Overall, 149 patients had refractory disease or relapsed, including 34/52 (65%) PB+ and 115/225 (51%) PB-. Median follow-up was 7 years (range 0.7 - 22.2 years). The 5-year progression-free survival (PFS) of treated 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.

E1138
TREATMENT PATTERNS OF PATIENTS WITH FOLLICULAR LYMPHOMA IN A LARGE US-INSURED DATABASE FROM 2010 TO 2014
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Background: Follicular lymphoma (FL) is the second most common type of non-Hodgkin’s lymphoma. While there are therapeutic options for patients with FL, it remains an incurable disease with conventional therapies. Furthermore, real-world treatment patterns for patients with FL are not well characterized in the literature.

Aims: To characterize the real-world treatment patterns by line of therapy (LOT) for patients with FL in a large US-insured database.

Methods: Using the Optum integrated database, patients with FL were identified and included if 1) they were diagnosed with the International Classification of Diseases, Ninth Revision (ICD-9) codes 202.0 or 202.00 to 202.08 between January 2010 and December 2014; 2) their age was ≥ 18 years at the index date (defined as date of FL diagnosis); 3) they received guideline-recommended treatment for FL, and 153 patients (6%) had steroids as first-line treatment, while 1180 patients (63%) received guideline-recommended therapy for FL. The median duration of follow-up was 610 days. Across all LOTs, 1180 patients (63%) were treated by rituximab in monotherapy, respectively 79% of early stage patients and 11% of the second LOT, respectively. Across all LOTs, the use of other FL treatments was very low, including rituximab-cyclophosphamide-vincristine-
E1139

A PHASE 1 STUDY EVALUATING THE SAFETY AND PHARMACOKINETICS (PK) OF VENETOCLAX (VEN) IN JAPANESE PATIENTS (PTS) WITH NON-HODGKIN LYMPHOMA (NHL) AND MULTIPLE MYELOMA (MM)


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Background: The antiapoptotic protein BCL-2 is commonly overexpressed in hematologic malignancies. VEN is a potent, selective, orally bioavailable BCL-2 inhibitor that has demonstrated acceptable safety and antitumor activity in NHL and MM pts.

Aims: To evaluate the PK profile, and preliminary antitumor activity of single-agent VEN in Japanese pts with NHL or MM.

Methods: Phase 1 open-label, dose-escalation study of VEN in Japanese pts with relapsed or refractory (R/R) NHL or MM (NCT02265731). Dose escalation followed a 3+3 design. After a 2-week ramp-up period with weekly dose escalation, VEN was administrated at daily final doses of 300, 600, 900, or 1200mg on 21-day cycles until progression. All pts received tumor lysis syndrome (TLS) prophylaxis (allopurinol, hydration, hospitalization and monitoring) starting at least 72 hours before the first VEN dose and before each dose escalation. Adverse events (AEs) were assessed by NCI CTCAE v4.0. Dose-limiting toxicities (DLTs) were determined during the ramp-up period and during cycle 1. Responses were assessed by 2007 IWG (NHL) or 2006 IMWG (MM) criteria.

Results: As of January 19, 2017, 20 pts (50% male; median age 65 years [39–81]) have been enrolled: 3 pts in the 300-mg, 7 pts in the 600-mg, 7 pts in the 900-mg, and 3 pts in the 1200-mg VEN dose cohorts. Eighteen (90%) pts had NHL (stage III/IV, n=14), including 11 with follicular lymphoma (FL), 6 with diffuse large B-cell lymphoma (DLBCL), and 1 with concurrent FL+DLBCL: 2 (10%) pts had MM at diagnosis. Treatment-emergent AEs (all grades) in >20% of pts included 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 (35%). One pt in the 600-mg cohort experienced dose-limiting thrombocytopenia as DLT after receiving 2 doses of 100-mg VEN on day 2 of the dose ramp-up period. One DLBCL pt died while on study due to disease progression. No TLS events were reported. Stable-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-Najapanese 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.

E1140

A SIMPLIFIED APPROACH IN THE ASSESSMENT OF T-CELL CLONALITY BY FLOW CYTOMETRY

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Background: T-cell lymphoproliferative disorders are among the most challenging diagnoses in haematology. Flow cytometric T-cell receptor (TCR)-β chain repertoire analysis (TCR-(V)β-R) is a sensitive method for detection of T-cell clonality; however, the assay is cumbersome owing to the required eight-part analyses that limit its clinical utility.

Aims: Here we describe a simplified flow cytometric method utilising a monoclonal antibody that targets the T-cell receptor β constant domain 1 (TRBC1). The cβ TCR is a pan T-cell antigen, expressed on >90% of T-cell lymphomas and all normal T-cells. A feature of the TCR is that the β-constant region comprises 2 functionally identical genes: TRBC1 and TRBC2. Each T-cell phenotype CD3+CD8+CD4-, CD3+CD4+CD8+, CD3+CD4+CD8- and CD3+CD4+CD8- respectively. The T-cell line, Jurkat was exclusively positive for JurA1. Of the 9 patients with T-LGL, 7 patients shared a common T-cell phenotype CD3+CD8+CD4-, one patient was predominantly CD4+ and the other patient was dual negative for CD4 and CD8. Jovi-1 expression within the normal T-cell population of this group of patients was >90% restricted to one compartment; these findings were confirmed by TCR-(V)β-R analysis. Similar results were also obtained in each case of T-NHL and Sezary syndrome, more than 90% of T-cells from the population with an abnormal phenotype (CD3dim/CD4+CD8-, CD3+CD4+CD8+, CD3+CD4+CD8- and CD3+CD4+CD8- respectively) were either positive or negative for Jovi-1. Patients with persistent lymphocytosis were also assessed for Jovi-1 expression in this group all patients had JurA1-positive and negative compartments within CD4 and CD8 T-cells.

Summary/Conclusions: In summary we have demonstrated a novel approach in the assessment of T cell clonality by targeting T-cell receptor β constant domain 1 (TRBC1). The addition of Jovi-1 in routine practice could improve the clinical evaluation of abnormal T-cell populations by flow cytometry.

E1141

A HIGHER AMOUNT OF LILOTOMAB PRE-DOSE INCREASES THE ACTIVITY-ADJUSTED AUC AND HAS A PROTECTIVE EFFECT AGAINST MYELOPRESERVATION OF LUTETIUM (177Lu)-LILOTOMAB SATETRAX-1 IN INDOLENT NHL PATIENTS

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Background: Lutetium (177Lu)- Elliotomab satetrax-1 (Lil), V-80714, is a novel CD37-binding murine IgG1 antibody radionuclide conjugate (ARC), in a ready-to-use formulation currently in Phase 1/2 clinical development for the treatment of non-Hodgkin lymphoma (NHL). Previously, pharmacokinetic (PK) data have been reported from 2 treatment arms of the ongoing LYMRT-37-01 study. In this abstract PK data from 4 treatment arms are presented for the first time.

Aims: This PK sub-study in INHL patients (pts) was designed to determine the PK profile of 177Lu-lilotomab when administered after different four pre-dosing schedules.

Methods: Patients with relapsed incurable indolent NHL, with platelet counts ≥150 x10⁹/L and <25% bone marrow involvement were eligible for inclusion in the study. All pts received either one or two doses of rituximab to deplete normal B cells. In addition, prior to 177Lu-lilotomab administration pts also received: - 40mg lilotomab prior to 10, 15 or 20 MBq/kg 177Lu-lilotomab; - no pre-dosing prior to 10 or 15 MBq/kg 177Lu-lilotomab; - 250 or 375mg/m² rituximab prior to 15 MBq/kg 177Lu-lilotomab; - 100mg/m² lilotomab prior to 10 or 20 MBq/kg 177Lu-lilotomab.

PK samples were collected at 0 and 5 minutes, then after 1, 2, and 20 hours and at 2, 3, 4, 7, 11 and 21 days post-177Lu-lilotomab administration. The patients were followed with weekly blood counts for 12 weeks post-treatment.

Results: A total of 22 pts were enrolled into this PK sub-study, 19 with follicular lymphoma, 2 with mantle cell and 1 with marginal zone histologies. The number

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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. Table 1 shows a summary of the median PK and haematology safety results for 177Lu-lilotomab by treatment group.

Aims: To investigate the potential for drug-drug interaction between OFA and BEN alone and in combination, along with the safety and tolerability assessments in patients with iNHL.

Methods: In this Phase I open-label, multicentre study, patients (aged ≥18 years) with previously 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 a 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 lymphoma patients with iNHL and therefore the potential for drug-drug interaction was investigated. Ofatumumab (OFA) alone or in combination with BEN exhibited manageable safety profile in patients with iNHL.

Summary/Conclusions: A higher pre-dose of lililotomab increases the activity-adjusted AUC and decreases the volume of distribution and clearance rate of 177Lu-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 lililotomab may have a protective effect against the myelosuppression associated with 177Lu-lilotomab. Further characterisation of 20 MBq/kg dose of 177Lu-lilotomab with 100mg/m2 of lililotomab 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
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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 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, AUClast, AUCinf were derived using non-compartmental analysis. All adverse events (AEs) and severe AEs (SAEs) were recorded for safety assessments.

Results: Thirty two patients were randomized (15 in Arm A and 17 in Arm B), 3 patients in Arm A discontinued study treatment due to consent withdrawal (2 patients) and infusion related AE (1 patient). All 32 patients were included for safety and PK concentration analysis while 30 patients (15 in each arm) were included for PK parameters. Patient and disease characteristics were similar between treatment arms; the majority of patients from both arms did not receive prior NHL therapy. PK concentration profiles and PK parameters of OFA were comparable when administered alone or co-administered with BEN (Table 1). As compared to OFA alone, there was a decrease of 14% in Cmax and 15% in AUClast when OFA was co-administered with BEN, which was not considered relevant (Table 1). BEN PK concentration profiles and PK parameters were comparable with or without OFA co-administration (Table 1). All patients reported AEs. The most frequent treatment-related AEs were infusion related reaction in 53% and 47%, nausea in 33% and 35%, fatigue in 33% and 18% patients in Arm A and Arm B, respectively. The percentages of patients with grade 3/4 AEs were higher in Arm A (53%) compared to Arm B (24%). Cytopenias were present in 40% of patients in Arm A and 6% in Arm B. Four SAEs were related to study treatment in Arm A while none in Arm B.

Summary/Conclusions: No relevant drug-drug interaction between OFA and BEN was observed in this study. OFA alone or in combination with BEN exhibited manageable safety profile in patients with iNHL.
**Infectious diseases, supportive care**

E1143

**ASSESSMENT OF INTERNATIONAL CONSENSUS GROUP FOR HEMATOLOGY (ICGH) SMEAR REVIEW RULES FOR AUTOMATED PLATFORMS IN THE DETECTION OF MALARIA**

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**Methods:**

Of the 153 samples included, all had *P. falciparum* parasitemia and 37 were collected from patients with severe malaria. A FBC with a DWCC was performed in 72/153(47.1%) patients, and a FBC alone in 81/153(52.9%). SR rules were triggered in 132(86.3%) patients (68(84.0%) in those with only a FBC and 64(88.9%) in those with a FBC and DWCC). The thrombocytopenia (platelets (Plt) <100x10^9/l) and anemia (Hb <7g/dl) rules were the most common, triggering in 105(79.5%) and 24(15.7%) patients respectively. Common analyzer morphology flags included those querying the presence of atypical lymphocytes, immature granulocytes and blasts, but 1/4 of these triggers were in 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.

**Results:**

Of the 153 samples included, all had *P. falciparum* parasitemia and 37 were collected from patients with severe malaria. A FBC with a DWCC was performed in 72/153(47.1%) patients, and a FBC alone in 81/153(52.9%). SR rules were triggered in 132(86.3%) patients (68(84.0%) in those with only a FBC and 64(88.9%) in those with a FBC and DWCC). The thrombocytopenia (platelets (Plt) <100x10^9/l) and anemia (Hb <7g/dl) rules were the most common, triggering in 105(79.5%) and 24(15.7%) patients respectively. Common analyzer morphology flags included those querying the presence of atypical lymphocytes, immature granulocytes and blasts, but 1/4 of these triggers were in 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, with 3% being 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 parasitemia was low (median 0.35% in those missed vs 3.1% in those with parasites identified). Reassuringly, SR rules were triggered in all the patients with severe malaria, and the parasites identified in 90.5% of these.

**E1144**

**A PROSPECTIVE MULTICENTER STUDY OF CANDIDEMIA IN NEUTROPENIC PATIENTS WITH HEMATOLOGICAL DISEASES: INCIDENCE, RISK FACTOR AND OUTCOMES**

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**Methods:**

A total of 1139 consecutive cases were enrolled in this study. Out of 1139 neutropenic cases, 8 developed candidemia. The median time from neutropenia to diagnosis of candidemia was 18 days (range: 8-20 days). Among the 8 cases with candidemia, 5 were neutropenic and 3 were critically ill. The cumulative incidence of candidemia in neutropenic patients with hematological diseases was 0.00% [95% confidence interval (CI): 0.00, 0.00%] at 7 days, 0.26% (0.00, 0.65%) at 14 days, 2.24% (0.67, 3.81%) at 21 days and 2.24% (0.67, 3.81%) at 28 days after neutropenia, respectively. Among 8 cases with candidemia, 3 were from patients receiving HSCT, other 8 were from patients who had acute myeloid leukemia (AML) and receiving induction chemotherapy.

The cumulative incidence of candidemia in patients with AML and receiving induction chemotherapy was also significantly higher than that in patients receiving HSCT and other patients (5.45% vs. 3.1% vs 0.00%, P=0.023).

**Summary/Conclusions:**

This study provided a description for the epidemiological study of candidemia in neutropenic patients with hematological diseases. This study defined the risk factors associated with candidemia in these patients, and confirmed that based on the risk factors, risk-stratification could identify the patients with a high-risk of candidemia.
fluid was studied by bacterial and fungal cultures, GM and PCR for Streptococcus pneumoniae, Legionella pneumophila, Chlamydophila pneumoniae, Mycoplasma pneumoniae, Bordetella pertussis, Bordetella parapertussis, Haemophilus influenzae, respiratory virus including CMV, Pneumocystis jiroveci, Mycobacterium tuberculosis complex, Nocardia spp., Lysteria monocytogenes and Aspergillus spp. Available commercial kits were used according to manufacturer’s instructions.

Results: Out of 769 patients consecutively admitted in our ward, 85 had LI and 47 of them underwent BAL (total amount: 51 procedures). A causal agent of LI was detected in 33 cases (65%) allowing to modify the ongoing antimicrobial treatment in 25 of these ones (76%). Twelve cases of LI fulfilling the radiologic criteria for IPA, though presenting only a positive Aspergillus PCR on BAL, were detected and treated as probable IPA. One life-threatening post-procedure complication was observed.

Summary/Conclusions: BAL seems a safe approach for an early diagnosis of LI in hematologic patients. The assessment of a broad diagnostic panel allowed the detection of a putative agent in 65% of cases. Assessment of Aspergillus by PCR on BAL proved useful for probable IPA diagnosis.

E1146
ESCape drug-resistant infections in hematological malignancies. DARE to Review!

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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 ESCAPE (Enterococcus, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter, Pseudomonas aeruginosa, Enterobacter). As suggested in recent literature, other common and difficult-to-treat infections such as Clostridium difficile and Enterobacteriaceae organisms (E. coli, Proteus) can be added to this group and change the acronym from ESCAPE to ESCAPE-A.

Aims: We performed a retrospective review of the rate of ESCAPE infections, resistance profile, and outcomes in patients with various hematological malignancies at the Houston Methodist Hospital from 2006 to 2015.

Methods: The patient data was obtained from METEOR (Methodist Environment for Translational Enhancement and Outcomes Research), a clinical data warehouse that contains records dating back to January 1, 2006, with over 3 million patients and over 10 million unique patient encounters. We queried for hematologic patients with hematological cancers at a high risk for increasingly resistant and severe infections. The Infectious Diseases Society of America has defined commonly resistant bacteria as ESCAPE (Enterococcus, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter, Pseudomonas aeruginosa, Enterobacter). As suggested in recent literature, other common and difficult-to-treat infections such as Clostridium difficile and Enterobacteriaceae organisms (E. coli, Proteus) can be added to this group and change the acronym from ESCAPE to ESCAPE-A.

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, 244 had CML, 39 had ALL, and 10 had an unspecified hematological cancer. Of 1132 infectious events, 62% were ESCAPE infections. The bacteria most frequently isolated were Enterococcus (23.4%), Staphylococcus aureus (18.5%) and Pseudomonas (16.9%). Bacteremia was the most predominant type of infection (41.9%) followed up by urinary tract infections (38.2%). Patients with MDS (39.6%) and AML (25.3%) were mainly affected. A prevalent resistance to levofloxacin was detected in gram positives and gram-negative organisms (29-54%). Pseudomonas, E. coli, Proteus and Klebsiella pneumoniae showed a significant resistance to broad-spectrum antibiotics including aztreonam (23-34%), cefepime (7-23%), and imipenem (22-39%). Pseudomonas had the highest mortality rate (45.2%), followed by Enterococcus (44.2%), and Pseudomonas (36.7%).

Summary/Conclusions: Hematological cancers with risk for neutropenia such as MDS and AML were the most affected by ESCAPE. 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 antimicrobial treatment in 25 of these ones (76%). Twelve 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.

E1147
Proposed Pegfilgrastim Biosimilar CHS-1701 Demonstrates Pharmacokinetic and Pharmacodynamic Similarity to Marketed Pegfilgrastim 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 (CPA)-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-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-∞ if the 90% confidence interval (CI) for the geometric mean ratio (GMR) was within 80-125% and for PD (ANCmax and ANC AUC0-∞) if the 90% CI for the GMR was within 80-125%.

Results: In the rat model, CHS-1701 and MP demonstrated similar time- and dose-dependent changes in the number of peripheral neutrophils and in the proliferative response in the bone marrow. No differences between CHS-1701 and MP in PD (Fig. 1) or PK were observed across the tested dose range. In the clinical study, PK bioequivalence criteria were met for Cmax (GMR=105.0; 90% CI 95.5, 115.4) and AUC0-∞ (GMR=97.5; 90% CI 88.6, 107.2). Pre-specified PD bioequivalence criteria (90% CI) and more stringent criteria (95% CI) were met for ANCmax (GMR=99.6; 90% CI: 96.2, 103.2; 95% CI: 95.5, 103.9) and ANC AUC0-∞ (GMR=96.7; 90% CI: 92.2, 101.4; 95% CI: 91.4, 102.4). The two treatments displayed similar safety profiles. Investigator-designated treatment-related AEs occurred in 71.9%, 71.2%, and 62.8% of subjects during the CHS-1701, first MP, and second MP dosing periods, respectively, and most commonly included back pain (46.9%, 42.3%, 30.8%), headache (29.2%, 36.9%, 29.5%), and arthralgia (8.3%, 13.5%, 7.7%). There were no treatment-related serious AEs.

Figure 1.

Summary/Conclusions: The dose-dependent changes in the neutropenia model were consistent with the PD effects of pegfilgrastim in humans and demonstrate that CHS-1701 results in comparable neutrophil recovery and time course compared to marketed pegfilgrastim. The clinical study demonstrates highly similar PK, PD, and safety profiles in humans for CHS-1701 and marketed pegfilgrastim. Overall, preclinical and clinical results suggest that CHS-1701 would provide similar PK, PD, safety, and efficacy to marketed pegfilgrastim in patients with chemotherapy-induced neutropenia.

E1148
A Retrospective Review Identifies a Resistant Microbial Strain, Antimicrobial Sensitivities and Risk Stratification of First Line Antibiotic Use in Adult Cancer Patients with Neutropenic Sepsis

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Background: Neutropenic sepsis remains a leading cause of morbidity and mortality in both haematology-oncology and general oncology patients on cytotoxic chemotherapy. Micafungin is a novel echinocandin approved as prophylaxis in haematology patients. We aimed to assess the efficacy and toxicity of micafungin in prophylaxis in neutropenic patients.

Methods: A retrospective review of patients treated for neutropenic sepsis was conducted for the period between 1/4/2015 to 11/10/2016. Analysis of potential risk factors including primary disease, age, sex, treatment regimen, albumin, neutrophil and lymphocyte count to assess potential association with adverse outcomes.

Results: There were 116 episodes of neutropenic sepsis in 92 patients in this period. Of these, 61 were haematology-oncology patients and 31 general oncology. 42 of 76 positive cultures identified gram-negative organisms. 40 patients received single agent Tazocin and 71 patients (61.2%) received Tazocin and an aminoglycoside as first line antibiotic treatment. Fourteen isolates demonstrated resistance, including 2 cases of stenotrophomonas maltophilia and 12 cases of enterobacteriaceae. 13 of the 14 resistant isolates were found in haematology-oncology patients. Nine of these cases were resistant to single agent Tazocin but sensitive to an aminoglycoside. The mean age of cases with resistance to Tazocin was 54.2 years (median 62). There was no significant difference in sex or degree of neutropenia/lipopenia in the cases that contracted resistant bacterial strains compared to those that were culture negative. Of the 4 fatal cases with resistant bacteria, 3 had low albumin (mean 25.5g/L cf. mean of 34g/L in resistant bacteria cases surviving).

Summary/Conclusions: This retrospective analysis supports the use of combination antimicrobials up front as first line treatment in high-risk patients with neutropenic sepsis. The study has demonstrated that the patient cohort most at risk of developing drug resistant bacteria are patients with high-risk or relapsed haematology-oncological disorders like AML or high-grade lymphoma, requiring multiple cycles of intensive chemotherapy. Of the patients who isolate resistant bacteria, identifying low albumin early may be a potential marker for adverse outcome in terms of morbidity and mortality. Of interest only one oncology patient isolates a resistant strain of bacteria, furthermore only 25% of general oncology patients treated with neutropenic sepsis had positive cultures compared to 75.8% of haematology-oncology patients. When comparing these findings to UK NICE recommendations it is clear that first line use of Tazocin in general oncology patients may well suffice in initial treatment of neutropenic sepsis. However with haematology-oncology patients early or up front consideration for the additional usage of an aminoglycoside is essential to optimize favorable outcome in 2012, UK NICE guidelines. 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 setting may support patient population and side close collaboration between clinicians and microbiologists is essential in providing optimal antimicrobial therapy algorithms in neutropenic patients.

E1150

USE OF MICAFUNGIN IN PROPHYLAXIS IN ONCO-HEMATOLOGY: RESULTS OF AN OBSERVATIONAL, MULTICENTER, PROSPECTIVE FRENCH STUDY (OLYMPRE)


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Background: Antifungal prophylaxis is being used increasingly.

Aims: The therapeutic arsenal is extensive and requires a better understanding of micafungin use in onco-hematology where most-at-risk patients of invasive fungal infections (IFI) are managed.

Methods: This observational study was conducted in 18 onco-hematology units in adult patients and children treated with micafungin in prophylaxis with a 3-months follow-up period.

Results: 150 patients (96 adults, 55 children) were included and represent the analysis population. In total, 15 patients (10%) presented an IFI during micafungin treatment. Among them, 11 presented a probable or proven IFI. The rate of IFI was higher in children (15%, n=8) than in adults (7%, n=7) and seem to have been influenced by the type of hematology and if the patient was allo-grafted or not: 13% (n=8) in allografted patients, 9% (n=4) in patients with AML or SMD and 7% (n=3) in other patients. Median time to infection was 24 days (1 to 68 days) and was longer in adults (25 days, 4 to 68 days) than in children (16.5 days, 1 to 68 days). Twelve patients (8 children and 4 adults) presented at least one clinical or radiological sign of suspected IFI. Fungus was identified in 8 patients (62%), mostly in blood cultures (50%, n=4); candidiasis in 4 patients, aspergillosis in 3 patients and infection related to Rhisopus in 1 patient. Incidence rate of IFI (10%, 5 patients) was inferior to prophylaxis failure rate (23%, 34 patients). Prophylaxis failure rate takes in account patients who switched to empirical treatment besides patients who switched to preemptive or curative treatment. After the end of prophylaxis, 4 patients (3 adults and 1 child) presented a proven IFI. Median time to infection after the end of treatment was 10,5 days in adults (7 to 24 days) and 52 days in children. Micafungin was overall well tolerated: only 10 patients (7%, mostly children) presented grade 1 to 4 adverse events related to micafungin, including 5 patients (3% with grade 3 or 4 adverse events).

Summary/Conclusions: Efficacy and safety profile of micafungin in prophylaxis are similar to what was observed in previous studies. Incidence IFI at 3 months confirms the clinical effectiveness of micafungin in prophylaxis in high risk patients. The low rate of serious adverse events confirms micafungin safety profile, in children included.

E1151

OUTBREAK OF MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA (MPA) IN A HAEMATOLOGY WARD (HW): MANAGEMENT AND INFECTION CONTROL MEASURES

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Background: Voriconazole is a triazole antifungal agent that has broad spectrum antifungal activity. It is widely used in the treatment of invasive fungal infections. However, there is limited information about its safety and effectiveness in hematologic patients who received voriconazole for the treatment or prevention of invasive fungal disease.

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 (Ctrough) 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 polymorphic isozyme, and 1 subject had a combination genotype. No significant difference was found between the two groups in the trough values (1.56±1.86μg/ml vs 3.30±2.35μg/ml; p=0.00). The Ctrough of the 45 patients were detected for 119 times totally. The medium of the Ctrough 45 hematological patients were 3.9±0.5μg/ml.

Summary/Conclusions: 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 important role in voriconazole metabolism and drug plasma level in setting of Asian population especially in hematologic patients is unknown. The hepatic cytochrome P450 isoenzyme 2C19 plays a important role in voriconazole metabolism and drug plasma level in setting of Asian population especially in hematologic patients is unknown. The hepatic cytochrome P450 isoenzyme 2C19 plays a important role in voriconazole metabolism and drug plasma level in setting of Asian population especially in hematologic patients is unknown.
Iron metabolism, deficiency and overload

E1154

GLYCOSYLATED FERRITIN MEASURING SIGNIFICANCE FOR SECONDARY HEMOPHAGOCYTIC SYNDROME DIAGNOSTICS

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Background: Hemophagocytic syndrome (HPS) is a clinicopathologic condition characterized by systemic inflammatory reaction with cytopenia and tissue damage. The HFS may be primary (genetic associated) or secondary (SHPS), caused by different systemic disorders (immune, infectious, neoplastic). The overall clinical symptoms are similar to sepsis, so it could be difficult to differentiate among these entities. Ferritin levels are high in both cases, but the glycosylated/nonglycosylated ferritin fractions ratio is seems to be indicative.

Aims: The estimation of the ferritin fractions ratio and biochemical profile in patients with sepsis and SHPS.

Methods: The data from 64 patients were analyzed: 40 pts with diagnosed SHPS (median age 57, range 8-74 years) and 24 with lethal septic shock (median age 57.5, range 18-52 years). SHPS in patients with persistent fever refrac- tory to antibacterial therapy and/or prolonged cytopenia and/or organ (lungs, CNS) involvement was established after the other conditions had been excluded. Sepsis diagnostics was based on the confirmed infection site and systemic inflammation with multiorgan failure. The following serum values were analyzed: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH), bilirubin, creatinine, INR, C-reactive protein (CRP), procalcitonin (PCT), total ferritin, and glycosylated ferritin percentage. Mann-Whitney U test and ROC-analysis were used for statistical analyses.

Results: No differences were found in sepsis and SHPS for ALAT, ASAT, AlPh, LDH, and bilirubin levels. The difference of INR, CRP, 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, AIPH, 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. Significant laboratory differences ($p$ values) between sepsis and SHPS.

Summary/Conclusions: The most difference between sepsis and SHPS was observed for triglycerides, ferritin and percentage of glycosylated ferritin. Percentage of glycosylated ferritin fraction seems to be the most indicative, which may make it useful for SHPS diagnostics and its differentiation from sepsis.

E1155

SERUM HEPCIDIN QUANTIFICATION IN INFLAMMATORY BOWEL DISEASES

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Background: Inflammatory bowel diseases (IBD) includes different intestinal pathologies, most common among them are 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 “Alek-sandrovska” hospital in Clinic of Gastroenterology. Their results were compared to age and gender matched healthy controls. Laboratory assessments were analyzed for included groups – iron, ferritin, CRP, IL-6 and hepcidin. AAS, nephelometric, ELISA and statistical methods were used during analyzes and obtained results interpretation.

Results: 53 from our patients had with iron deficiency anemia (IDA) and low hepcidin concentrations (5.9±1.1 µg/L) compared to control group (19.9±2.8 µg/L). P<0.001. 11 of included patients had combination of chronic disease (ACD). Their hepcidin levels were increased (59.9±4.6 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: Quantity 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 ANEMIA WITHOUT SHOWING EVIDENCES OF MYOPATHY AND LACTIC ACIDOSIS

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Background: Mutations in the gene YARS2 encoding mitochondrial tyrosyl-tRNA synthetase have previously been identified as a cause of MLASA2, a mitochondrial respiratory chain disorder presenting with myopathy, lactic acidosis and congenital sideroblastic anemia (OMIM #610957, OMIM #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.

Aims: Here we 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_001040436.2 c.[1104-1G>A], and a missense variation NM_001040436.2 c.608 G>T, which are on-going to confirm pathogenicity of the novel missense variation.

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 consider 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 DYSERYTHROPOIETIC 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 some of the patients may have mild disorder whereas others may be transfusion dependent. The ineffective erythropoiesis and the transfusional iron load puts these patients at risk for iron overloading and there is very scarce data on the iron loading and chelation types in these patients.

Aims: We aimed to summarize the chelation results of our patients with CDA from a single center.

Methods: Of the 33 patients with CDA, 11 were initiated iron chelation treatment either for receiving more than 20 packed RBC transfusions previously or for having serum ferritin levels above 1000ng/ml.
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 HEMOCHROMATOSIS 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 (JJH), a rare genetic disorder inherited with an autosomal recessive manner, resulting from mutations in either the hemochromatosis HJV (type 2A) or the hepcidin (HAMP) gene (type 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 erythrocytapheresis.

Aims: The scope of this study was to evaluate the use of an oral iron chelation therapy in young children with HH.

Methods: 3 children (2 females and 1 male) were diagnosed with HH with the age of 4, 6 and 8 years old, respectively, based on increased ferritin and transferrin saturation indexes and exclusion of other causes of hyperferritinemia. Genetic analysis were performed in all 3 patients and showed positive results in 2 of them, while on the 3rd no genetic changes could be identified. All patients were at pre-symptomatic stage of the disease and they were referred for evaluation after hyperferritinemia was discovered on a routine screening examination. Liver iron concentration (LIC) and cardiac iron concentration were evaluated by MRI (table 1). Iron chelation therapy with deferasirox (DFX) at low dose (10 mg/kg/24h) was initiated, after evaluation was completed and permission from regulatory authorities obtained.

Table 1. Clinical characteristics of the patients.

<table>
<thead>
<tr>
<th>Age (years) at treatment</th>
<th>Gene Type</th>
<th>Ferritin at treatment (ng/ml)</th>
<th>Transferrin saturation (%)</th>
<th>LIC (mg/g dry wt)</th>
<th>Cardiac iron T2* (ms)</th>
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<tbody>
<tr>
<td>1</td>
<td>HJV-HJV</td>
<td>728</td>
<td>50%</td>
<td>1.4</td>
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</tr>
<tr>
<td>1</td>
<td>HJV-HJV</td>
<td>360</td>
<td>11%</td>
<td>0.6</td>
<td>3.7</td>
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<tr>
<td>2</td>
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<td>3.7</td>
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</table>

Results: All 3 patients responded promptly to therapy and showed decreased levels of ferritin, LIC and cardiac iron concentration. Gastrointestinal disturbances were noted in 1 patient, which resolved with H2-blockers and with changing the treatment to 5d/wk (patient 2). Mild increase in serum creatinine (>33% from baseline but within normal range for her age) was observed in patient 3, which resolved with temporary cessation of the chelation therapy.

Summary/Conclusions: Although the mechanism of neuropsychiatric hypersegmentation in iron deficiency anaemia is not clear, it is thought that iron acts as a cofactor in folate metabolism and / or DNA synthesis in granulocytes. There are a limited number of studies dealing with NH associated with ID in the literature. However most of these studies were observational and did not include controls or were not blinded. Our study is the first to demonstrate the association of NH with iron deficiency anaemia in adults in the absence of megaloblastic anemia.

E1159

NEUROPHIL HYPERSEGMENTATION IN ADULTS WITH IRON DEFICIENCY: A CASE-CONTROL STUDY

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Background: Neutrophil hypersegmentation (NH) has been accepted as a hallmark of the macrocytic anaemias associated with the deficiency of cobalamin or folate. However, there are a small number of reports stating that NH might accompany iron deficiency anaemia. The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia).

Aims: The aim of the present study was to determine the association of NH with iron deficiency (with or without anaemia) in adults and also to compare neutrophil segmentation status in anaemia group before and after oral or parenteral iron treatment.

Methods: Fifty-six patients with iron deficiency and 20 age and sex matched controls were included in this prospective, single blind, case-control study between February-November 2016. Subjects were included if they were ≥ 18 years of age, and had normal serum vitamin B12 and folate levels, liver, thyroid and renal function tests. Pregnant women and patients with a history of blood transfusion within last 3 months and/or those with acute renal failure, anaemia of chronic disease, hypothyroidism, additional cytopenias and infection were excluded. Patients with iron deficiency were divided into 2 groups being with iron deficiency anaemia (IDA) and iron deficiency without anaemia (ID). Those who were anaemia were further evaluated prior and after iron replacement. Results of the study groups were compared to age and sex matched healthy controls. Blinded peripheral blood smear slides were evaluated by a haematologist by counting 200 neutrophils. Hypersegmentation was defined as reported by Bain et al. Iron deficiency was diagnosed based on the findings of iron parameters including serum iron, total iron binding capacity, and ferritin. Anaemia was defined according to the WHO recommendation. Cohort characteristics were given in Table 1.

Table 1.

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</table>

Results: Hypersegmentation was detected in 25 individuals with iron deficiency (45%) and 1 healthy control (5%). It was significantly more frequent in the IDA group (48.8%) than in the ID group (30.7%) [p<0.001]. After iron treatment 3 IDA patients’ peripheral blood smear were checked and with normalization of iron parameters and hemoglobin, hypersegmentation was undetectable. The study is still ongoing and rest of the IDA group are still on treatment, their peripheral blood smears are to be examined after iron treatment is over.

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)-taclidomus (calcineurin inhibitor) as GVHD prophylaxis. Drug doses were adjusted according to blood levels and renal function. Levels of Hb, MCV and iron parameters were also systematically evaluated after alloRIC. Microcytosis was considered when MCV was below 80 fl.

Results: At 6 months 56 out of 61 (92%) were alive. Anemia was observed in 30 (49%) of them, with only 8 cases (13.1%) showing Hb level below 100 g/l.

Microcytic anemia was diagnosed in 2 of them (3.3%). One patient showed an iron deficiency anemia due to gastrointestinal bleeding (Hb 94 g/l, MCV 69 fl, serum ferritin 21 µg/l). However, the second one, a 61-year old male with an acute leukemia, had a microcytic anemia with iron parameter changes similar to those observed in kidney transplantation and associated with increased hepcidin, (see table). Anemia progressively improved with sirolimus tapering.

Table 1.

| Inh: inhibitors, Hb: hemoglobin, MCV: mean corpuscular volume, Fe: iron, TIBC: total iron binding capacity,%SAT: % transferrin saturation, Bm: bone marrow, Nv: normal values. |

Summary/Conclusions: In contrast to kidney transplantation, microcytic anemia related to immunosuppression with mTOR inhibitors was seldom observed in alloRIC recipients. However, this association should be taken in account in this setting, as a rare cause of anemia. In case of microcytic anemia, the evaluation of iron parameters and hepcidin provides the diagnosis of this rare type of anemia.

E1161

IRON METABOLISM IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Background: Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired clonal non-malignant hematological disorder that is associated with hemolytic anemia, immune-mediated failure, thrombosis. At the onset, the condition is often interpreted as iron deficiency anemia that leads to the prescription of ferotherapy.

Aims: Study iron metabolism in patients with PNH.

Methods: The study group included 19 patients (11 men and 8 women aged from 20 to 64 years, median age 43 years) with a diagnosis of PNH, followed up in our Center between 2014 and 2017. The median hemoglobin level was 8.1 g/dl. The erythrocyte PNH clone size ranged from 17 to 99%, median - 47%. Granulocyte and monocyte PNH clone sizes were 85% and 89%, respectively. The following parameters were studied to characterize iron metabolism: ferritin, transferrin, iron concentration, total iron binding capacity (TIBC), transferrin saturation. In addition, we also analyzed iron distribution under intravenous and oral iron supplementation. Furthermore, we investigated the activity of iron absorption under oral iron supplementation in mice as well as under intravenous iron supplementation. In addition, we also analyzed iron distribution under intravenous and oral iron supplementation.

Results: Serum iron levels in IV and Oral iron groups were significantly higher compared with control group. However, serum iron levels were elevated only in oral iron group. In immunohistochemical analyses, expression levels of duodenal DMT1 were not detected in all groups and expression levels of duodenal FPN in IV and Oral iron groups were significantly lower than control group. As for iron distribution in liver, iron was accumulated in reticuloendothelial cells in IV iron group, on the other hand, in Oral iron group iron was accumulated in parenchyma. In hematological analyses, although red blood cell and reticulocyte count were not significantly different among all groups, Ret-He and MCH in Oral iron group were higher than IV iron groups.

Summary/Conclusions: It was demonstrated in this study that serum iron levels were elevated in spite of high hepcidin levels and down-regulation of duodenal iron transporters under oral iron supplementation. Furthermore, iron was distributed in liver parenchyma and hemoglobin contents in each reticuloocyte and erythrocyte were up-regulated only under oral iron supplementation. We speculated that high serum iron lead to excess iron uptake into tissues and erythrocyte fraction. These data might provide an opportunity to rethink the importance of proper use of iron supplemements.

E1163

DEFERASIROX FOR SEVERE ANAEMIAS IN YOUNG CHILDREN

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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 haematology 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 where evidence is sparse. Our patients were aged 1-36 months of age. Deferasirox was started at the age of 2 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 stem cell transplant at the age of 3 years old. Transfusion and deferasirox were subsequently stopped.

Figure 1.

Summary/Conclusions: All of these children had stabilization or improvement of ferritin values after initiation of deferasirox as shown in figure 1. Deferasirox is licensed in Europe to be used in children with thalassaemia older than 6 years of age or older than 2 year of age when desferoxamine therapy is inappropriate or inadequate. Deferasirox is preferable in severe anaemias due to better side effect profile on the bone marrow compared to deferoxprone; the use of which can cause agranulocytosis or neutropenia. Furthermore, its oral administration improved compliance compared to desferrioxamine 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 ANEMIA. AN OBSERVATIONAL, PROSPECTIVE, MULTICENTRIC STUDY
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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 check points at 3 days (WBC and reticulocytes only), 2 weeks, 8 weeks, and at 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 ages 0-3 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 9/68 (13%) in the bis-glycinate iron group, in 3/19 (16%) of the elemental iron group, and in 0/12 of the liposomal iron group. Suspension of therapy due to side effects was needed only in 5 patients, 4 in the bis-glycinate and 1 in the elemental iron group, respectively. Final outcome was available for 77 patients; it was recorded as solved IDA, persistent IDA, or lost at follow up. Solved cases were 40/53 (75%) in the bis-glycinate iron group, 3/19 (16%) in the elemental iron group, and 8/13 (62%) in the liposomal iron group. Persistent cases were 8/53 (15%) in the bis-glycinate iron group, 6/11 (55%) in the elemental iron group, and 1/13 (8%) in the liposomal iron group. Lost at follow up were 5/53 (9%) in the bis-glycinate iron group, 1/11 (9%) in the elemental iron group and 4/13 (31%) in the liposomal iron group.

Summary/Conclusions: The collected data show that both bis-glycinate and liposomal iron formulations have a good efficacy/safety profile and offer a sustainable alternative to classic elemental iron preparations.

E1165

AN INVESTIGATION ABOUT WEIGHT GAIN WITH TREATMENT OF IRON DEFICIENCY ANEMIA: CHANGES OF GHRELIN AND HEPcidin LEVELS WITH TREATMENT
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Background: Iron deficiency anemia (IDA) is a global health problem and problems in compliance with oral iron therapy are frequently seen. It has been shown that medications are not used regularly or discontinued due to weight problems during the treatment process.

Aims: We investigated ghrelin, known as appetite hormone and its relationship with hepcidin, the homeostatic regulator of intestinal iron absorption, in order to explain some symptoms of IDA and weight gain during iron treatment.

Methods: A hundred and twenty adult IDA patients, referred to our clinic between October 2015 and October 2016 were included in the study. The study was completed with 87 patients, who gave the informed content and a control group consisted of 50 healthy people. Information about age, gender, weight, height, body mass index (BMI), waist-hip circumference and blood samples were taken from the patient and control groups. The treatment of IDA was done according to the dose and method recommended by the responsible physician, including basal assessment of whole blood count, reticulocytes, iron status, with subsequent check points at 3 days (WBC and reticulocytes only), 2 weeks, 8 weeks, and at 6 months. Clinical data regarding compliance to therapy, unwanted effects, final outcome were recorded.

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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.
were examined once in the control group and twice in the patient group, before and after treatment.

**Results:** When the patient and control groups were compared, there was no significant difference in terms of age, sex, height, weight, BMI, waist and hip circumference. The pretreatment plasma hepcidin and ghrelin levels of the patient group were significantly lower than in the control group, showing a significant increase with treatment, but it was not associated with weight gain. Interestingly, these neurons showed a positive but weak correlation with the increase in ghrelin levels of those people. Hepcidin plasma levels increased compared to the pretreatment levels (80 ± 21 ng/dl vs 164 ± 150 ng/ml, p < 0.001). Although an increase in the plasma ghrelin levels was encountered after treatment, it was not statistically significant (152 ± 119 pg/ml vs 164 ± 150 pg/ml, p = 0.589). When correlations of individual increases in ghrelin levels were examined, a weak positive correlation was found between increase in ghrelin levels and weight gain.

**Summary/Conclusions:** In our study, ghrelin was significantly lower than the control group in the IDA group, suggesting that it may be the cause of loss of appetite. Ghrelin is also detected in neurons of hypothalamic arcuate nucleus, which regulates appetite. The deficiency of iron may cause deficiencies in enzymatic activities of iron-dependent enzymes and it may disturb the function of these neurons. But the increase with treatment did not reach statistical significance. This may be due to physiological suppression of levels by weight gain. When patient-based weight gains were examined, there was a positive but weak correlation with the increase in ghrelin levels of those people. Hepcidin plasma levels increased compared to the pretreatment levels (80 ± 21 ng/dl vs 92 ± 13 ng/ml, p < 0.001). Although an increase in the plasma ghrelin levels was encountered after treatment, it was not statistically significant (152 ± 119 pg/ml vs 164 ± 150 pg/ml, p = 0.589). When correlations of individual increases in ghrelin levels were examined, a weak positive correlation was found between increase in ghrelin levels and weight gain.

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an epigenetic aberrant paradigm possibly involving transcriptional and transla-
tional regulation in JMML.

Methods: CD34+ cells isolated from 3 JMML patients samples collected at diag-
nosis (t0) and after the third cycle of AZA (t1) were evaluated together with those
of 3 healthy donors (HD). JMML patients have been treated with AZA on a com-
passionate use basis after obtaining signed informed consent. DNA samples
were processed and ion fragment libraries were prepared. MBD-seq, bioinfor-
matics and statistical analysis were performed by Genomnia srl (Bresso, Italy).

Results: First, we compared t0 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 t1 and HD cells. In
this comparison, 644 unique transcriptional units, including 468 coding and 175
non-coding sequences, were found. Hypermethylation in JMML samples com-
pared to HD was detected, but, unexpectedly, 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
methylation regions are shared between t0 vs HD and t1 vs HD sets. More in
detail, 261 and 15 coding regions and 107 and 10 non-coding regions were
uniquely found in t0 vs HD and t1 vs HD sets, respectively. However, 439 coding
and 161 non-coding genomic regions preserve their hypermethylated status,
probably due to a mechanism of resistance to AZA treatment. Among non-cod-
ing elements, we found different RNA species, such as microRNAs, splicing
RNAs, lincRNAs/antisense transcripts (AS) and other unknown RNAs. Retro-
transposons, belonging to LINEs and SINEs families, were also screened. We
identified 13 sequences with a significant differential methylation profile in both
t0 and t1 groups. 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.

Summary/Conclusions: In conclusion, the whole genome MBD-seq per-
formed for the first time on JMML CD34+ bone marrow derived cells, showed
a broad genomic hypermethylation both in pre- and post-AZA samples com-
pared to HD, suggesting a patient-specific AZA-effect. Transcription and trans-
lation processes of coding and non-coding genes could be deregulated in mul-
tiple ways, due to heterogeneity of sequences involved in CpG islands hyper-
methylation. Moreover, due to their known ability to insert random mutations in
the genome, retrotransposons should be candidate for further studies in JMML
pathogenesis.

E1168
RESPONSE MONITORING IN MDS WITH DEL(5q) USING DIFFERENT
FLOW CYTOMETRIC (FCM)-SCORES IN COMPARISON TO CYTOGENETICS
AN EMLNET IMDS-FLOW EXPERIENCE

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Background: Flow cytometry (FCM) is one part of integrated MDS diagnostics.
Different well established FCM-scores are applied, as FCSS (Wells et al. 2003),
Ogata-score (Ogata et al. 2012), new iFS (Cremers et al. 2017), and del(5q)-
FCM-score (Oelschlaegl et al. 2015).

Aims: The aim of this prospective study was to test, which of the mentioned
FCM-scores fits best for response monitoring in del(5q) MDS in comparison to
cytogenetics.

Methods: Overall, 245 FCM investigations were performed in 61 patients with
MDS and del(5q) (IPSS-R very low/low: n=26, int: n=13, high/very high n=22)
including 42 patients with isolated del(5q) or one additional cytogenetic abnor-
mality. The majority of analyses were performed in patients receiving lenalido-
amide or azacitidine (n=29 and n=22 patients), or in patients receiving
cytarabine chemotherapy and/or allogeneic transplantation or growth factors (n=10):
Standardized FCM (lyse-stain-wash) and cytogenetics/FISH procedures were per-
formed according to ELN guidelines at the TU of Dresden, VUMC of Amster-
dam, UH of Guadalajara and UH of Bristol. Cytogenetics/FISH analysis was
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 88% and a specificity
of 85%. This was confirmed if only MDS with del(5q) was 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 con-
sidering almost only abnormalities of the myeloid progenitors, ended up with a
slightly lower sensitivity (86%) and specificity (81%). The new iFS analyzing
progenitor cells, granulo-, mono- and erythropoiesis showed a comparably
high specificity (83%) but a slightly impaired sensitivity (72%). FCSS, analyzing
dyspoiesis of multiple cell lineages, showed a response in less than the half of
all investigations being in cytogenetic CR (sensitivity: 41%), but revealed a high
specificity (91%). The analysis of Hi-E was high sensitive (81%) but not as spe-
cific (62%). Next, we investigated the potential prognostic impact of response
monitoring using various FCM-scores compared to cytogenetics. Considering
all del(5q) MDS patients as well as only those patients with del(5q) as a single
abnormality, cytogenetics and all tested FCM-scores showed a significantly
longer OS for MDS responding to therapy. The highest prognostic impact dis-
played the new iFS (p=0.0019) and Ogata-score (p=0.0092), respectively. Eval-
uating only MDS treated with lenalidomide, response monitoring using FCSS
separated best the OS curves (p=0.0080). Finally, we combined the evaluation
of Hi-E with cytogenetics or the FCM-scores. This resulted in an even better
prognostic impact for the combination of Hi-E plus the new iFS (p=0.0010).

Summary/Conclusions: Flow cytometry might serve as a rapid tool for
response monitoring during treatment with disease-modifying drugs. All estab-
lished FCM-scores allowed for an at least similar correctness of response pre-
diction. The prognostic impact of the various FCM-scores seems to be even
higher than that of cytogenetic response evaluation in this MDS subgroup. One
reason might be, that most of the FCM-scores reflect not only the genetic back-
ground of the MDS but dyspoietic alterations in various cell lineages of the
hematopoietic system.

E1169
EVALUATION OF MUTATIONS AT RELAPSE IN MYELODYSPLASTIC
SYNDROME PATIENTS RECEIVING ALLOGENIC STEM CELL
TRANSPLANTATION

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Background: Allogeneic transplant (AlloSCT) is the only curative therapy for
myelodysplastic syndromes (MDS). Unfortunately, relapse is the main cause of
treatment failure. Evaluation of genetic mutations both at diagnosis and

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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 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 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 4570 reads (range 1450-6873). 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 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 epigenetic and stem cell pathways on two cell lines: AML (BW90), MDS (MDS-L) and on pt bone marrow samples.

**Background:** Azacitidine (AZA) is the standard of care for patients (pts) with higher-risk MDS, however, only 50% of pts respond and the majority will relapse within 2 years. All pts ultimately fail treatment due to primary or secondary resistance. RIGosertib (RIG) is a “ras mimetic” agent that binds to the Ras Binding Domain of RAF kinases and inhibits the RAS-RAF-MEK and PI3K pathways. Initial results of an ongoing Phase III study with RIG combined with AZA or vorinostat (VOR) in patients with MDS demonstrated a response rate of 76% (90% in pts following hypomethylating agent (HMA) failure) and 85% in HMA naïve pts (Navada et al ASH 2016).

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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 TINF2 germline variant 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 experiments showed that the new TINF2 mutation reduced TIN2 binding with TRF2 in vitro.

Summary/Conclusions: A new TINF2 germline variant 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 experiments showed that the new TINF2 mutation reduced TIN2 binding with TRF2 in vitro.

E1175

FUNCTIONAL EXPRESSION OF TIM-3 AND CLINICAL SIGNIFICANCE OF PLASMA GALACTEIN-9 LEVELS IN MYELODYSPLASTIC SYNDROMES

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Background: T-cell immunoglobulin and mucin domain-3 (Tim-3) is an inhibitory immune checkpoint molecule that suppresses adaptive immunity by binding with galectin-9 (gal-9). The Tim-3–gal-9 pathway is associated with self-renewal and cell proliferation. We compared 145 bone marrow samples from 58 women and 87 men, aged from 26 to 85 suspected to have myeloid dysplasia were investigated. Of these only 76 (52%) were found to fulfill the WHO criteria referred to as MDS positive, the rest as MDS negative. Gene variances were detected in all but 7 samples (42%). The latter as expected were devoid of gene mutations. We observed driver mutations as reported in myeloid malignancies in 68 (47%) samples whilst 70 (48%) were found to carry the same variations seen in disease free individuals or of unknown significance. As expected driver variances were not identified in any of the samples that failed the WHO criteria for MDS. Variance were detected in all samples for 35 of the 54 genes targeted by the TruSight panel. In order of frequency these were TET2, SRSF2, ASXL1, CUX1, DNMT3A, RUNX1, BCR and HRAS, seen in more than 10% of all samples, while the rest were less frequently reported. The aberrant genes ASXL1, TET2 and SRSF2 figured prominently in both groups of samples with comparable frequencies as may be expected from published data and there were in addition a number of aberrant genes unique to the MDS group albeit at low frequency. When we examined the distribution of individual variances (rather than genes) we found a number of unique loci of the genes ASXL1 at 131022441, U2AF at 131021822 and TET2 at 106192728 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 patients presenting with MDS of which 76 met the WHO criteria. There is little difference in their genomic profile when comparing the two groups on the basis of the most highly involved genes (ASXL1, TET2 and SRSF2) but if we compare the two groups by variance, 9 variances are exclusively associated with MDS positive disease.

E1177

SUPPRESSION OF DNA METHYLTRANSFERASE ENZYMES BY A NOVEL HYPOMETHYLATING AGENT, SG-1027, AND AZACITIDINE IN DECITABINE-RESISTANT CELL LINES

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Background: Myelodysplastic syndromes are a collection of clonal hematopoietic disorders with a wide range of clinical manifestations and eventual outcomes. Predicting the prognosis is of great importance for defining the risk and choosing appropriate therapeutic options. Among risk factors of poor stratification are, all of which include genetic markers along with other clinical and paraclinical features. The Revised International Prognostic Scoring System (IPSS-R, Greenberg et al., Blood. 2012;120(12): 2454-2465) defines 5 risk levels based on the presence of specific chromosome abnormalities. These genome aberrations provide evidence for disease (p<0.008) and reports of frequent driver mutations (Papamorga et al., Blood, 2013) and/or structural variants detected by single nucleotide polymorphism (SNP) arrays (Tiu et al., Blood, 2011) have shown a potential for score criteria in the diagnosis of MDS. Recent reports of the presence such genetic aberrations in disease free individuals makes this approach problematic (Genovese et al., N Engl J Med 2014; Lichman, Blood 2015, Kwok et al., Blood 2015). A study of patients without evidence for MDS identified a driver mutation and/or structural gene variants in 91% of pre-diagnostic samples with the mutational spectrum mirroring that seen in MDS population (Cargo et al. Blood, 2015). The presence of mutations with greater median variant allele fraction (40% vs 9% to 10% in healthy individuals) and occurring with additional mutations (>2 mutations, 64% vs 8%) were shown to define a high-risk group with a shorter time to disease progression and poorer overall survival.

Aims: To compare the genomic profile of bone marrow from 145 adults, 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 54 cancer-related genes relevant in myeloid malignancy. Gene variances were reported at allele frequencies (VAF) >10% and at minimum read depth of 300 as per manufacturers criteria. We used the Catalogue of Somatic Mutations In Cancer (COSMIC), dbSNP and 1000 genome (≥2%) to classify gene variants as either drivers, variants of unknown significance and germline polymorphisms (SNPs).

Results: A total of 145 bone marrow samples from 58 women and 87 men, aged from 26 to 85 suspected to have myeloid dysplasia were investigated. Of these only 76 (52%) were found to fulfill the WHO criteria referred to as MDS positive, the rest as MDS negative. Gene variances were detected in all but 7 samples (42%). The latter as expected were devoid of gene mutations. We observed driver mutations as reported in myeloid malignancies in 68 (47%) samples whilst 70 (48%) were found to carry the same variations seen in disease free individuals or of unknown significance. As expected driver variances were not identified in any of the samples that failed the WHO criteria for MDS. Variance were detected in all samples for 35 of the 54 genes targeted by the TruSight panel. In order of frequency these were TET2, SRSF2, ASXL1, CUX1, DNMT3A, RUNX1, BCR and HRAS, seen in more than 10% of all samples, while the rest were less frequently reported. The aberrant genes ASXL1, TET2 and SRSF2 figured prominently in both groups of samples with comparable frequencies as may be expected from published data and there were in addition a number of aberrant genes unique to the MDS group albeit at low frequency. When we examined the distribution of individual variances (rather than genes) we found a number of unique loci of the genes ASXL1 at 131022441, U2AF at 131021822 and TET2 at 106192728 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 patients presenting with MDS of which 76 met the WHO criteria. There is little difference in their genomic profile when comparing the two groups on the basis of the most highly involved genes (ASXL1, TET2 and SRSF2) but if we compare the two groups by variance, 9 variances are exclusively associated with MDS positive disease.
Methods: Besides azacitidine and decitabine, three other agents (SGI-1027, zebularine, and gemcitabine) are known as having hypomethylating effect. In vitro activities of the 5 HMA’s on HMA resistant cell lines (MOLM/AZA-1 and MOLM/DEC-5) were tested by cell viability assay using luminescent-based CellTiter-Glo system. Protein and mRNA levels of DNMT enzymes (1, 3A, and 3B) were assayed before and after treatment of each HMA. Proteosomal degradation and activation of Akt were also determined to see the correlation with changes of DNMT’s.

Results: Although azacitidine and decitabine could suppress DNMT1 and DNMT3A in MOLM-13, the agents could not suppress DNMT enzymes in resistant cell lines. Inhibition of proteosomal degradation by bortezomib induced accumulation of DNMT enzymes in MOLM-13, whereas it did not accumulate the enzymes in MOLM/AZA-1 and MOLM/DEC-5. Phosphorylated Akt (p-Akt) was dramatically overexpressed in MOLM/AZA-1 and MOLM/DEC-5. SGI-1027 showed the lowest IC50 values for MOLM/AZA-1 and MOLM/DEC-5, and it suppressed the protein levels of all three DNMT enzymes. SGI-1027 could also decrease the level of p-Akt. GDC-0941, a PI3K inhibitor, suppressed DNMT1 and DNMT3A as well as p-Akt, but it could not decrease DNMT3B in MOLM/AZA-1 and MOLM/DEC-5. Cell viability assay showed the synergistic effects of combination of GDC-0941 and Nanomycin A, a specific DNMT3B inhibitor, in MOLM/AZA-1 and MOLM/DEC-5.

Summary/Conclusions: DNMT levels of MOLM/AZA-1 and MOLM/DEC-5 were not dependent on proteosomal degradation. DNMT1 and DNMT3A might be regulated via PI3K-Akt pathway, while regulation of DNMT3B might be different from DNMT1 and DNMT3A. SGI-1027 appears to exert inhibitory effects on MOLM/AZA-1 and MOLM/DEC-5 by inhibition of both p-Akt and DNMT3B.

E1178

MECHANISTIC HIGHLIGHTS OF IMPROVED ERYTHROPOIESIS WITH A LOW DOSE OF DEFERASIROX IN LOW RISK MYELODYSPLASTIC SYNDROMES

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Background: Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal stem cell disorders leading to ineffective hematopoiesis. Anemia is a frequent cytopenia in MDS and the majority of patients requires red blood cell (RBC) transfusion resulting in the development of iron overload (IO). Deferasirox (DFX) became a standard treatment of IO in MDS and seems to have positive effects on hematopoiesis with a reduced need of RBC transfusion.

Aims: Decipher the mechanisms of the potential improvement of erythropoiesis with DFX.

Methods: We report our in vitro data about the proliferation, cell cycle, apoptosis, erythroid differentiation, and cell signaling pathways concerning CD34+ hematopoietic stem progenitor cells from low risk MDS samples in a 2-step erythroid differentiation liquid culture with low dose DFX and iron overload.

Results: We observed a higher proliferation rate for cultures with 3µM DFX versus the control condition (p=0.038). In contrast, no increased proliferation was found with DFX>5µM and with other chelators used in the clinic. The higher proliferation rate with DFX 3µM was due to the combination of decreased apoptotic cells at day 10 (D10) (p=0.03) and D14 (p=0.007) and increased cycling cells at D10 (p=0.0001). Regarding clonogenic assays, there were more CFU-E colonies with DFX 3µM (p=0.04). Despite the low concentration of DFX, cells expressed in DFX 3µM had a lower intracellular iron concentration measured by ICP-MS than control cells (p=0.019). Nevertheless, this decreased iron amount was not sufficient to activate cellular iron regulation by Iron Regulatory Proteins suggesting the absence of a direct effect of low dose DFX on iron homeostasis. Moreover, low dose DFX decreased intracellular and mitochondrial reactive oxygen species (ROS) at D14 (p=0.048 and p=0.03) and decreased the level of malonaldehyde (p=0.048), a product of lipid peroxidation. Then, we have investigated which signaling pathways were sensitive to DFX 3µM. We found an increased nuclear translocation of NFκB detected by both CM (p=0.04) and luciferase reporter assay (p=0.03). NFκB activation was absent in the knock-down (KD) of mitochondrial TRX (siTRX2) condition. Moreover, in non-iron overloaded medium condition, the level of ROS was not increased, and DFX in the TRX1 KD condition was not associated with NFκB activation. These results suggest that NFκB activation in this model is linked to TRX1 and regulated by an extremely fine control of ROS levels with a likely threshold effect.

Summary/Conclusions: Our study describes the pro-proliferative effects of low dose of DFX on erythroid progenitors in low risk MDS patients. These results provide a biological rationale for a clinical trial which will propose low dose of DFX in MDS patients, refractory to erythropoiesis stimulating agents.
E1179
EVALUATING ERYTHROBLAST PAS POSITIVITY IN THE DIAGNOSTIC APPROACH OF MYELODYSPLASIC SYNDROME

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 their degree of PAS reactivity. The discriminant power of both PAS positivity rate and score for MDS identification was evaluated in comparison with that of the conventional morphological features of dyserythropoiesis; then, PAS positivity was included into the morphological scoring system we have previously defined (Leukemia 2015;29:66-75).

Results: PAS positive erythroblasts were observed in 104 (63%) MDS patients, 46 (40%) patients with non-clonal cytopenia, and 12 (24%) non-cytopenic 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 internuclear bridging (r=-0.23, p=0.0395). A ROC curve analysis allowed us to identify a PAS score value ≥1 (AUC=0.697, p=0.0008) and a PAS positive erythroblast percentage ≥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. Integrating conventional parameters and PAS results significantly improved the sensitivity of our morphological scoring system.

Summary/Conclusions: The evaluation of BM erythroblast PAS positivity, easy to perform and inexpensive, may be useful in the work-up of patients with suspected MDS, especially if there is only 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 MEYLODYSPLASTIC SYNDROMES (MDS)

Background: There is a lack of PBO-controlled data for erythropoiesis-stimulating agents (ESAs) in MDS.

Aims: To evaluate darbepoetin alfa (DAR) in IPSS low/int-1 risk MDS (EudraCT2009-016522-14, NCT01362140).

Methods: Patients with MDS per WHO 2008 criteria with IPSS low/int-1 risk, anaemia [hemoglobin (Hb)≤10 g/dL], low transfusion burden, no previous treatment with ESAs or biologic response modifiers, and serum EPO≤500mU/mL were randomized 2:1 to 24 weeks (wk) SC DAR 500 µg or PBO every 3 wk (Q3W), stratified by IPSS, then 48 wk open label (OL) DAR follow-up is ongoing. Doses were withheld for Hb>12g/dL and decreased if Hb increased by >1.5 g/dL in 3 wk. Key endpoints were transfusion incidence and Hi-E per IWG 2006.

Results: Randomized patients [N=147] had median Hb of 9.3 (min-max:5.5-10.6) g/dL and median baseline EPO of 69 (min-max:4.3-497) mU/mL. WHO classification was RA:15%, RARS:14%, RAEB-1:16%, RAEB-2:16%, and MDS-U/unknown:2%. Transfusion incidence wk 5-24 was significantly reduced with DAR [DAR:36.1% vs PBO:59.2%, p=0.008]. In the 48-wk OL DAR period, 50.8% of patients had transfusions. More DAR patients achieved Hi-E in the double blind period [DAR:14.7% (11/75 evaluable) vs PBO:0% (0/35 evaluable), p=0.016]. In the 48-wk OL DAR period, 34.7% (34/98) of patients achieved Hi-E. Improved Hi-E and transfusion responses were seen with more favorable status for IPSS-R but not IPSS. In the 48-wk OL DAR period, dose frequency increased from Q3W to Q2W in 81% of patients; doses were held/reduced frequently. Safety results from this trial were consistent with the previous DAR phase 2 MDS trial, with similar AML rates in PBO and DAR arms.

Figure 1. Summary/Conclusions: In this phase 3, randomized, double-blind, PBO-controlled trial in anemic IPSS low/int-1 risk MDS patients, 24 wk of darboepoetin alfa Q3W significantly reduced transfusions and increased Hi-E rates with no new safety signals. Most patients met criteria to change to Q2W dosing during the 48-wk OL period, suggesting that Q2W dosing may offer more benefit. The true clinical benefit of darbepoetin alfa may have been underestimated due to the nature of IWG 2006 Hi-E criteria and trial design (Hb measured Q3W, dosing rules).
Background: Lenalidomide (LEN) is the first choice of treatment in low risk MDS patients with isolated del(5q) (MDS-del(5q)) and transfusion dependency (TD). Most of the low risk MDS-del(5q) patients diagnosed with anaemia and independent of transfusions developed TD or needed treatment for symptomatic anaemia early after diagnosis (median of 20 months, abstract 3180.ASH, 2016). LEN directly targets the del(5q) clone improving anaemia, quality of life and survival in these subset of patients. For these reasons, the use of LEN in patients with del(5q), anaemia and not TD seems to be very attractive. However, data about the use of LEN in MDS 5q- patients and transfusion independency (TI) are scanty, some retrospective studies suggest a benefit with the early use of LEN in this setting, but there is not already available any prospective and randomized study to confirm this likely advantage.

Aims: Our aims were to analyze efficacy and safety at week 12 of treatment with LEN vs Placebo in this setting of low risk MDS del(5q) patients with anaemia and not in TD at diagnosis

Methods: From 2010 to 2017, 47 patients have been included in the Sintra-Revl trial, a phase III, multicenter, randomized and double blind study with LEN (5mg/day) vs placebo [2:1 randomization] in Low – Int-1 risk (IPSS) MDS del(5q) patients with anaemia but TI. Preliminary results of efficacy (according to the IWG 2006 criteria for erythroid [HI-ER] and cytogenetic response [CyR]) and safety has been analyzed at week 12. Progression disease (DP) in the trial was defined as the development of TD.

Table 1.

<table>
<thead>
<tr>
<th>Age median (range)</th>
<th>72 (37-89)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M/F</td>
<td>7/40</td>
</tr>
<tr>
<td>Platelets</td>
<td>263 x 10^9</td>
</tr>
<tr>
<td>Neutrophils median</td>
<td>1.2 x 10^9</td>
</tr>
<tr>
<td>iPSS (R in %)</td>
<td></td>
</tr>
<tr>
<td>very low</td>
<td></td>
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<tr>
<td>Low</td>
<td></td>
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<tr>
<td>Intermediate</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>CyR (in %)</td>
<td></td>
</tr>
<tr>
<td>Del(5q) alone</td>
<td>39 (83%)</td>
</tr>
<tr>
<td>Del(5q) + other</td>
<td>36 (73%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (10%)</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 (5 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 42% of these were relevant (G3-4). Most G3-4 AE were hematological (neutropenia 38%) being non-hematological only in 42% of these were relevant (G3-4). Most G3-4 AE were hematological (neutropenia 38%) being non-hematological only in 42% of these were relevant (G3-4).

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.
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) was performed. MFC 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) was performed. MFC 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) was performed. MFC 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) was performed.

**Table 1.** HCU and Cost (Overall Year 1) for Patients Treated with HR-MDS

<table>
<thead>
<tr>
<th>Category</th>
<th>MDS-related</th>
<th>Non-MDS-related</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Admission</td>
<td>11,050 (SD: 12,976)</td>
<td>12,976 (SD: 14,135)</td>
<td>24,026 (SD: 27,111)</td>
</tr>
<tr>
<td>Consultation</td>
<td>12,976 (SD: 14,135)</td>
<td>14,135 (SD: 15,905)</td>
<td>27,111 (SD: 30,080)</td>
</tr>
<tr>
<td>Test</td>
<td>27,111 (SD: 30,080)</td>
<td>30,080 (SD: 33,915)</td>
<td>57,191 (SD: 64,095)</td>
</tr>
</tbody>
</table>

**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**

**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 identified in these patients, possibly contributing to both bone marrow insufficiency and immune-related cytopenia in a series of 20 consecutive patients with MDS at a single institution.

**Methods:** 17 patients with refractory anemia (RA) and 3 patients with RA plus excess blasts (RAEB) were included in this study. All patients were treated with intravenous immunoglobulin (IVIG) at a dose of 400 mg/kg/week for a minimum of 3 months. A complete hematologic response was defined by the absence of peripheral cytopenia and an increase in peripheral blood counts.

**Results:** A complete hematologic response was achieved in 12 patients (60%), with a median duration of 6 months (range 3-12 months). The remaining 5 patients showed a partial hematologic response. There were no serious adverse events associated with IVIG therapy.

**Summary/Conclusions:** IVIG appears to be an effective treatment for immune-related cytopenia in patients with MDS, with a high rate of complete hematologic responses. Further studies are needed to confirm these findings and evaluate the long-term benefits of IVIG in the management of MDS-related immune-related cytopenia.

**E1186**

**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**

**Background:** 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.

**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-10 coding: ≥1 inpatient or ≥2 outpatient claims with ≥1 HR-MDS ICD-9/10 code (ICD-9 code: 234.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: lack of continuous enrollment in medical/pharmacy benefits in the 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during baseline period. MDS-related HCU and costs were medical claims with ≥1 HR-MDS ICD-9/10 code (ICD-9 code: 234.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date.

**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.
(3), seronegative arthritis (2), connectivitis (2). One patient with hypoplastic MDS had LGL liver involvement. Coombs test was positive in 4/16 hemolysis cases. From Jan-10 to Jan-17, IVIG was administered at a dose of 500mg/kg once per week, in cycles of 1 to 4 weeks. The ORR was 75% (15/20); all patients showed an erythroid hematological improvement (H1) (100%). Platelets and neutrophil H1 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 were 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: 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 Ce 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 pancytopenia associated with T-cell immune-clones and MDS was safe and may be considered a standard of care. The median OS and CR rates were 98% and 100%, respectively. 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 included 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).

E1186 DEVELOPMENT AND EXTERNAL VALIDATION OF A NEW PATIENT-CENTERED PROGNOSTIC INDEX FOR PATIENTS WITH ADVANCED MYELODYSPLASTIC SYNDROMES

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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 rate. We used a lower IPSS category. All completed a baseline health-related quality of life assessment. Data from international and DFCI cohorts were independently collected and analyzed. Univariate and multivariate Cox proportional hazards (PH) regression analyses were performed to estimate hazard ratios with 95% Confidence Intervals (CIs). Discrimination and calibration were evaluated for both the development (internal validation) and independent DFCI datasets (external validation). Statistical significance for all tests was set as two-sided α=0.05.

Results: A new risk classification was developed, namely, the fatigue (FA)-IPSS(h). Whereas use of the standard IPSS in more advanced disease discriminates between two risk categories for untreated patients, the new fatigue FA-IPSS(h) classification is able to distinguish three survival subgroups in patients with distinct survival outcomes. Overall survival rates at 6 months, 1 year, and 2 years were markedly different among the three groups. To illustrate, one year survival was 80.3% (95% CI, 73.4;87.8), 60.5% (95% CI, 52.3;70.0) and 37.6% (95% CI, 23.9;59.1) for patients classified in Risk-1, Risk-2 and Risk-3 respectively. Median OS in DFCI data by FA-IPSS(h) risk was similar to that of the development cohort for each of the three risk groups, indicating good external calibration. Patterns of OS through 2 years were also distinct between risk groups as in the development cohort of untreated patients, with one exception: 2-year OS was similar for FA-IPSS(h) risk 3 and risk 2. Predictive accuracy of this new index was higher than the IPSS alone in both the development cohort (C-statistic, 0.56 vs 0.57) as well as in the independent cohort including pre-treated patients (C-statistic, 0.58 vs 0.54).

Summary/Conclusions: The FA-IPSS(h) is an additional prognostic tool that might enhance clinicians’ ability to provide more personalized treatment strategies both in untreated and pretreated advanced MDS patients. This analysis offers a model for integration of PROs in prognostic systems for patients with other cancers and advanced illnesses.

E1187 PROGNOSTIC AND THERAPEUTIC IMPLICATIONS OF SIGNIFICANT MARRROW FIBROSIS IN COMBINATION WITH P53 OVER-EXPRESSION IN PATIENTS WITH MYELODYSPLASTIC SYNDROME: A SINGLE CENTRE STUDY

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Background: Myelodysplastic syndromes (MDS) are defined in the WHO 2016 classification as a group of clonal bone marrow neoplasms characterized by ineffective hematopoiesis, morphologic dysplasia in hematopoietic cells and peripheral cytopenia(s). They present as a diverse phenotype with some patients requiring merely observation while others require more intensive management due to significant marrow failure and the risk of development of acute leukemia. The presence of significant marrow fibrosis has previously been shown to be a poor prognostic factor in patients with MDS, with reduced overall survival. Significant marrow fibrosis has also been associated with both the presence of the TP53 gene mutation and with p53 over expression, which is a known adverse prognostic risk factor in MDS patients.

Aims: To assess the presence of p53 expression in patients with moderate to severe marrow fibrosis (grade 2-3), observe its effect on overall survival in patients with marrow fibrosis, and determine whether the use of azacitidine had any impact on survival.

Methods: We conducted a retrospective study utilizing a hospital database of 247 patients with MDS diagnosed in a single center between 2000 and 2014. Of these patients, 200 had bone marrow trephine samples adequate for reticulin stain analysis, which was completed using the European consensus on grading bone marrow fibrosis (grades 0-3). P53 expression was examined using immunohistochemistry staining techniques according to the modified quick scoring system. We then looked for an association between degree of marrow fibrosis and p53 expression. In patients with significant marrow fibrosis and p53 expression we examined overall survival and response to treatment with azacitidine.
Results: Overall, no significant correlation was seen between expression of p53 and degree of fibrosis (p<0.05). 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, those with negative significant fibrosis and p53 expression had a significantly increased overall survival compared with those who did not receive azacitadine (4 month versus 1 month, p=0.002). Azacitadine treatment was not associated with increased survival in patients with p53 expression without fibrosis but these patients did have an overall increased survival compared to those with fibrosis. 

Summary/Conclusions: This study confirms that significant narrow 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 was lacking, our 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

E1188

FACS PURIFICATION OF BLAST CELLS IN MDS IMPROVES THE FISH DETECTION RATE FOR DEL(5Q) AND DEL(20Q), BUT NOT FOR DEL(7Q) OR T8

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Background: Prognostication in Myelodysplastic Syndromes (MDS) using validated scores includes the detection of chromosomal aberrations by conventional karyotyping. When the latter is unavailable or unsuccessful, fluorescence in-situ hybridization (FISH) panels can be used. Although panels vary by laboratory, some of the commonly used probes include the search for monosomy 5 or del(5q), monosomy 7 or del(7q), del(20q) and trisomy 8 (T8). In our laboratory, 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 significantly doubled in FACS compared to Full Sample (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).

We 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 identified in 7.0% 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 10.2% of both full and FACS samples, p=NS. The percentage of positive cells once again doubled from 25.5±14.7% in the full sample to 53.3±28.1% after FACS (p=0.0008).

Summary/Conclusions: We found that one quarter of all patients who underwent a FISH panel workup for a suspected diagnosis of MDS presented with aberrations in at least one of the four selected probes, a proportion which was significantly lower (one fifth) when a full sample was analyzed, and significantly higher (one third) in FACS purified blast cells. Although the purification of the sample through FACS doubled the percentage of positive cells within each sample for all four probes, the likelihood of obtaining a positive result for del(7q) and T8 in the cohort was unaffected by the methodology used. In contrast, the use of a sorted sample greatly increased the proportion of positive findings in del(5q) and, especially, in del(20q), the two probes for which the basal positivity in full samples were lowest. The clinical value of this increased rate of detection of del(5q) and del(20q) remains unclear, since their prognostic utility has only been established for levels detectable by conventional karyotyping of a full sample.

E1189

COUNTING BONE MARROW BLASTS AS A PERCENTAGE OF NON-ERYTHROID CELLS PROVIDES SUPERIOR RISK STRATIFICATION FOR MDS PATIENTS WITH ERYTHROID PREDOMINANCE

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Background: Patients with erythroid predominance (≥50% erythroblasts, MDS-erythroid) compose a significant proportion of patients with MDS. The erythroid/myeloid subtype was divided from the AML category into MDS-erythroid by the 2016 WHO classification of myeloid neoplasms. At that time, there was no consensus on a more appropriate way of enumerating bone marrow (BM) blasts from TNCs or NECs in MDS-erythroid patients.

Aims: To clarify these questions, 1283 MDS patients were retrospectively analyzed in our center.

Methods: MDS-erythroid was observed in 27.0% of patients (346/1283), and these patients had similar clinicopathological features and overall survival, with 10% cases of MDS with <50% ENCS.

Results: By calculating the percentage of BM blasts from NECs, 73 of 200 patients (36.5%) with MDS-erythroid who were diagnosed within WHO subtypes without excess blasts (EB) were moved into higher-risk categories and showed shorter OS than those who remained in the initial categories (P=0.041). Recalculating the International Prognostic Scoring System-Revised (IPSS-R) by enumerating blasts from NECs, 40 of 168 (23.8%) MDS-erythroid patients with relatively lower risk were re-classified as higher-risk and had significantly poorer survival than those who remained in the lower-risk category (P=0.030). This was especially true for the intermediate risk group that was stratified by IPSS-R (unchanged patients vs shifted patients, P=0.007). However, the impact of enumerating BM blasts from NECs on classification and prognostication was not evident in all MDS patients.

Summary/Conclusions: In conclusion, our results suggested that enumerating the percentage of BM blasts from NECs significantly improved the prognostic assessment of MDS-erythroid, especially for patients within the intermediate risk group stratified by IPSS-R.

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-severe AA (8), severe AA ineligible/refractory to immunosuppressive therapy or allogeneic transplantation (3), transfusion dependent (TD) lower risk MDS refractory to conventional treatment (11), MDS with isolated thrombocytopenia <50x10^9/L (6) or with bone marrow hypoplasia and bicytopenia (3). Diagnosis was defined by WHO 2008 for MDS and according to Camitta (Blood 1975) for AA; response was assessed by IWG 2006 criteria.

Results: The characteristics of the patients are shown in the Table. All MDS and AA 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: hematotoxicity (3 G1, 1 G2, 3 G3), muscle pain/CPK elevation (3 G1, 2 G2), transient renal impairment (1 G1), hypoxemia (1 G1). Responders to danazol had a better survival in terms of OS and EFS in both groups (Figure 1).

Table 1.

<table>
<thead>
<tr>
<th>PFS from Initiation of 1LT</th>
<th>OS by Int-1LT</th>
<th>Total Int-1LT</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>13.3 (9.1, 17)</td>
<td>11.7 (9.1, 17)</td>
<td>0.02</td>
</tr>
<tr>
<td>Complete Response</td>
<td>14.1 (9.1, 19)</td>
<td>11.7 (9.1, 17)</td>
<td>0.02</td>
</tr>
<tr>
<td>Partial Response</td>
<td>3.4 (2.3, 5.3)</td>
<td>3.5 (2.3, 5.3)</td>
<td>0.93</td>
</tr>
<tr>
<td>Relapsed</td>
<td>0.4 (0.2, 0.9)</td>
<td>0.4 (0.2, 0.9)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: Danazol was proved both effective and safe as treatment of cytopenia in MDS and AA patients refractory or ineligible to standard therapies. The daily dose of 600mg was more effective for MDS patients, whereas a lower dose of 400mg may have a better risk/benefit ratio in AA. Younger AA patients with less severe anemia were more likely to respond. Danazol use is particularly attractive in thrombocytopenic patients, where responses were rapid, but delayed responses may be expected also on anemia by using dana-zol for prolonged periods, when tolerated. Response to danazol is also 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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1Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Pharmaceutical Company Limited, Cambridge, 2Xcenda, Palm Harbor, United States

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 coding: ≥1 inpatient claim with an HR-MDS ICD-9 code (ICD-9 code: 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-10 code: C91.9, C91.0, C91.1). OS was calculated from index date of 1LT to last follow-up or date of death, censoring at last contact or end of study. EFS was calculated from index date to date of first event (death, end of continuous enrollment, end of study, or progression to acute myeloid leukemia (AML)).

Results: 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 128:1124)

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 one hour intravenous decitabine infusion (IV-DAC) in a dose-escalation phase 1 study.

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 ASTX777, PD were assessed with LINE-1 methylation as measured on blood cells at baseline and days 8, 15, 21 and 28 in cycles 1 and 2. Full PK assessments of ASTX777 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 ASTX777 and IV-DAC was created for each patient. Safety and clinical response were assessed on all patients.

Results: 50 patients were randomized, 50 had matched PK and 46 had matched PD sample sets for the first 2 cycles. No significant differences were seen when comparing the randomized sequences for any parameters, so all assessments comparing ASTX777 and IV-DAC were performed independent of sequence. The geometric mean maximum demethylation was 9.9% for ASTX777 and 8.9% for IV-DAC (Ratio of oral over IV=0.9, with a lower limit of 90%CI of 0.8). The geometric mean AUC for IV-DAC was 161 ng/hmL. The 5 day total geometric mean of the AUC (ng/hmL) was 769 for ASTX777 and 805 for IV-DAC ([Ratio of oral over IV=0.96, with a lower limit of 90%CI of 0.806). Decitabine Cmax was higher for IV-DAC (189 ng/mL) than after ASTX777 (210 ng/mL) and the AUC0-5 was lower for IV-DAC (95.1 ng-dL/hmL). The Day 8 adverse events regardless of grade or causality were febrile neutropenia 34%, neutropenia 28%, thrombocytopenia 16%, fatigue 16%, and hypomagnesia 16%. There were no reported GI Adverse Events greater than Grade 2 with ASTX772 regardless of relationship to treatment.

Summary/Conclusions: Patients treated with azacitidine (especially AML patients) at high risk of infection treated with the first-line AZA are at risk. All important infection predictive factors should be assessed before therapy. Patients possessing factors predictive for infection require special approach and predictive infection model should be developed in further analysis.

E1194

OVERALL SURVIVAL, INITIAL TREATMENT AND TREATMENT DURATION OF PATIENTS WITH MYELODYSPLASTIC SYNDROME, A DETAILED POPULATION BASED STUDY

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Background: Population-based studies on myelodysplastic syndrome (MDS) containing detailed clinical information of patient characteristics, treatment and follow-up of the disease are scarce. Since 2005, all patients diagnosed with hematological malignancies in Friesland, a province in the Netherlands, are prospectively registered and followed by their clinicians in a population-based registry, the HemoBase. The registry provides representative population-based data on diagnosis, treatment and outcomes in an era where low-intensity treatment such as hypomethylating agents have become available for the elderly.

Aims: The objectives of this study were to determine the overall survival (OS) of patients with MDS and the effect of the variables gender, age, comorbidities, IPSS-R score and MDS subtype according to WHO 2016 classification. Furthermore, the leukemia free survival (LFS), the initial treatment and the duration of first-line treatment were analyzed.

Methods: An observational, population-based study was performed using the HemoBase registry. The bone marrow biopsies and aspirates of all MDS patients were assessed between 01-01-2005 and 31-12-2013 were independently and blindly reviewed by both the hematologist and hematologist-pathologist and classified according to the WHO 2016. Treatment categories were defined as intensive chemotherapy (IC) either combined or not combined with allogeneic stem cell transplantation, the hypomethylating agent azacitidine, the immunomodulatory agent lenalidomide, hydroxyurea or best supportive care (BSC) (blood transfusions, erythropoiesis-stimulating agents). Approval was obtained from the Medical Ethics Review Committee from Medical Centre Leeuwarden. Statistical analyses were performed with SPSS 19; survival analyses were used during Kaplan-Meier estimates.

Results: 217 patients (72.4% male, 66.8% >70 years old, median age 75 years, 27.2% Charlson Comorbidity Index (CCI) score ≥3) were diagnosed in a median follow-up duration of 70.2 months. 15.7% of the population had an IPSS score ≥15 and 12.4% of the population had an IPSS-R score ≥4.5. In 41.5% no cytogenetic information was available. MDS-RS, MDS-SLD/-MLD, MDS-EB, MDS-U and CMMl were diagnosed in 11.5%, 14.7%, 36.4%, 27.2% and 10.1% of the population respectively. 18.4% showed progression towards acute myeloid leukemia (AML), IC, azacitidine, lenalidomide, hydroxyurea and BSC were the initial treatment in 5.1%, 13.8%, 1.4%, 9.7% and 66.4% of the patients respectively. Within 12 months 78.1% of all treated patients terminated their first-line therapy because of death (20.0%), refractory to treatment (18.3%) or disease progression (16.7%). A second treatment was initiated in 10.1% of patients. The median LFS was 18.2 months (95% CI: 12.6-23.8). The median OS of MDS patients in Friesland was 22.5 months (95% CI: 15.2-29.7). Univariate analysis showed an association between lower OS and male gender (HR for women: 0.54, p=0.008, 95% CI: 0.34-0.85), age >80 years (HR: 2.7, p=0.0005, 95% CI: 1.6-4.6), CCI score ≥3 (HR: 2.0, p<0.001, 95% CI: 1.3-3.0), IPSS score ≥1.5 (HR: 2.3, p=0.004, 95% CI: 1.3-4.1), IPSS-R score ≥4.5 (HR: 5.7, p<0.0005, 95% CI: 2.4-2.4) and MDS subtype MDS-EB (HR: 1.8, p=0.016, 95% CI: 1.1-2.9).

Summary/Conclusions: This study provided complete representative population-based data on overall survival and treatment of patients with MDS. IPSS-R score, treatment sequence and MDS subtype according to WHO 2016 classified according to the WHO 2016. Despite the low-intensity treatment strategy that was used in 72.5% of the patients and significant comorbidity in this population, a third of the patients received treatment in addition to BSC.

E1195

DANAZOL TREATMENT FOR THROMBOCYTOPENIA IN LOWER-RISK MYELODYSPLASTIC SYNDROMES: A REAL LIFE EXPERIENCE

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Background: Severe thrombocytopenia is an uncommon event in lower-risk MDS patients, but it may significantly affect the prognosis. In fact, when it occurs, the need for antiplatelet therapy may be a life-threatening situation. Danazol, a synthetic androstenedione, is a naturally effective progestin. Danazol treatment for thrombocytopenia is nowadays available yet for this unmet need in Europe. Eltrombopag seems to be a very interesting product, but its efficacy and safety still need to be better demonstrated. Even romiplostim could be
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 diagnosis.

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.

Figure 1.

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 (HMA) (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 code (ICD-9 code: 238.73; ICD-10 codes: D46.20, D46.21, D46.22). The first MDS claim served as the index date. Exclusion criteria included: absence of continuous enrollment in medical and pharmacy benefits for 12 months prior to index date (baseline period), presence of other primary cancer, or receipt of any chemotherapy or SCT during the baseline period. First-line therapy (1LT) was defined as an MDS-specific treatment (as defined by NCCN MDS Guidelines v2.2017) initiated on or after the index date. Patients were followed until death, end of continuous enrollment, or end of study (12/31/2015). For patients with progression to acute myeloid leukemia (AML), treatment pattern evaluation stopped at AML diagnosis.

Results: 335 newly diagnosed HR-MDS patients were identified; 209 (62.4%) were treated with 1LT with treatment initiated within 1 month of diagnosis (median: 17 days, interquartile range [IQR]: 9, 35). A higher proportion of untreated patients (n=126) was ≥75 years of age (71.4% vs 66.2%, p = 0.03), and had certain comorbidities at baseline (congestive heart failure, 23.0% vs 16.3%; renal disease, 24.6% vs 16.3%; diabetes 31.0% vs 23.4%, diabetes with end organ failure, 16.7% vs 8.1%) than treated patients. For treated patients, 1LT with azacitidine predominated in 68.9% of patients (n=144), followed by decitabine in 20.6% of patients (n=43), and immunomodulators (lenalidomide or thalidomide) in 8.7% of patients (n=19) (Figure 1). 4 patients had only SCT and an additional 14 had SCT at some point during follow-up. With regard to HMA therapy, median duration was 4.5 months (IQR: 2.6, 9.5) for azacitidine and 4.8 months (IQR: 2.1, 11.6) for decitabine. A greater proportion of decitabine-treated patients...
received supportive care with colony-stimulating factors (CSFs) (39.5% vs 28.5%) and either erythropoietin or platelet transfusions (69.8% vs 57.6%) during 1LT vs azacitidine-treated patients. Second-line therapy (2LT) was administered to 30 (14.4%) patients; the HMAs again predominated in 63.3% of patients (n=19). Of patients not receiving 2LT, 65 (31.7%) progressed to AML, 47 (22.9%) had <30 days of follow-up due to proximity to end of study (38 [80.9%] of these were on 1LT at end of study). 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

APPRIC8: A PIPELINE FOR PRECISE VARIANT CALLING INTEGRATING 8 TOOLS

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Background: For the use of next-generation sequencing in clinical routine same platform, on a different platform and expert-based review. (42,322 bp) 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 on Illumina HiSeq, and 111 MDS patients, sequenced on 1LT at end of study. Almost 100% of the genome was analyzed. From this data, 33 (16.1%) continues 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.

E1198

COMPARISON OF ADMINISTRATION OF HYPOMETHYLATING AGENTS AND EFFICIENCY OF ALLOGENEIC SCT IN ELDERLY PATIENTS WITH ADVANCED MDS

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Background: Hypomethylating agents (HMA) have been introduced as a promising agent in the treatment of elderly patients with advanced myelodysplastic syndromes (MDS) leading to a response in approximately 50% of patients. However, most of the patients relapse and estimated years survival is below 10%. Stem cell transplantation (SCT) still represents the only curative treatment even in elderly patients with advanced MDS and it is connected with long-term survival in 35-40% despite relatively high risk of transplant related mortality (25-30%).

Aims: The aim of the study was a retrospective analysis of results of the treatment of 59 elderly patients (50 years of age or older) with MDS RAEB-2 or with acute myeloid leukemia with multilineage dysplasia with less than 30% of bone marrow blasts (MDS RAEB-T according to the FAB classification) who received either HMA or underwent allogeneic SCT.

Methods: In the HMA group, 34 out of total 38 patients received azacitidine (Vidaza®) in the dose of 75mg/m²×7 every 28 days and 4 patients were treated with decitabine (Dacogen®) in the dose of 10mg/m²×5 every 28 days. Median number of cycles administered was 10.4 (range 3-31). An age and diagnosis matched transplanted group consisted of 21 patients, 9 patients were transplanted upfront, 12 patients were pretreated either with combination chemotherapy (10 patients) or with HMA (2 patients) and achieved CR prior to SCT. Ten patients received myeloablative conditioning and 11 patients were transplanted after reduced conditioning regimen.

Results: When analyzing the training sets with only one of the eight variant calling programs and considering all variants pathogenic as well as somatic, sensitivity ranges between 0.85 and 1.00 in case of set 1 and 0.47 and 0.99 in case of set 2. Although FreeBayes features highest sensitivity regarding both sets, it consistently features lowest PPV as well (set 1: 0.03, set 2: 0.02). Combining the output of all variant calling tools leads to perfect sensitivity, while PPV is 0.03 for set 1 and 0.02 for set 2. Application of our appric8 pipeline led to a minor decrease in sensitivity (set 1 and set 2: 0.98), while PPV is significantly increased (set 1: 0.99, set 2: 0.94). The PPV of the appric8 output for both training sets is higher compared to each of the individual tools. Analysis of the independent test set 1 leads to comparable results. Sensitivity of the individual tools ranges between 0.82 and 0.99, while PPV ranges between 0.02 and 0.91. Combining the output of all variant calling tools leads to sensitivity of 1.00 and PPV of 0.02. However, application of appric8 leads to variant calling results with sensitivity of 0.98 and PPV of 0.09. 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 appric8 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 appric8 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.

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 MYELODYSTROPHIC SYNDROMES

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Background: Rigosertib, a novel phosphoinositide 3 kinase pathway inhibitor, induces G2/M arrest leading to the apoptosis of cancer cells and myeloblasts and is safe for and well tolerated by pts with low, intermediate-1, intermediate-2, or high-risk myelodysplastic syndromes (MDS).

Aims: The aims of the study were to assess the safety, efficacy, and pharmacokinetics of oral rigosertib and to determine the recommended dose (RD) for a Phase II clinical study in Japanese pts with recurrent/relapsed or refractory MDS.

Methods: We conducted a multicenter, open-label, Phase I clinical study of oral rigosertib. The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: ≥ 20 or older; ECOG PS of 0 to 2; and no major organ dysfunctions. Rigosertib (280 and 560mg BID) was administered orally in one 21 day cycle (up to cycle 6) that consisted of the 14-day, twice-daily, oral administration term, followed by 7-day monitoring. The primary endpoint was dose-limiting toxicity (DLT). The secondary endpoints were 1) safety as assessed with adverse events (AEs) and laboratory results, 2) efficacy as assessed with the International Working Group 2006 criteria, and 3) pharmacokinetics.

Results: Between March 2013 and November 2014, 6 male and 3 female pts (median age: 70; range 52-80) were enrolled. ECOG PS was 0 in 7 pts and was 1 in 2 pts, and 3 and 6 pts were eventually assigned to the 280 and 560mg BID arms, respectively. According to the FAB classification, 4, 2, 2 and 1 pts were categorized to RAEB, RARS, RA, and RAEB-1, respectively. The prognostic factor according to IPSS was Int-1 risk in 4 pts (1 and 3 pts in the 280 and 560mg BID arms, respectively) and was Int-2 in 5 pts (2 and 3 pts in the 280 and 560mg BID arms, respectively). DLT occurred in 1 pt in the 280mg BID arm and in 2 pts in the 560mg BID arm: the former consisted of type 2 diabetes and grade 4 delirium, and the latter grade 5 urinary tract infection and grade 3 prolonged QT interval. Therefore, the RD for a Phase II clinical study in Japanese pts was determined to be 560mg BID. On day 11 of treatment, 1 pt in the 560mg BID arm died of grade 5 urinary infection whose relationship with the investigational drug was rated to “Definite”. The presumed cause of death was septic shock caused by urinary tract infection. The hematological remission rate was 11.1% (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 gammopathies - Biology

NON-OVERLAPPING PROMOTER AND SUPER-ENHANCER DRIVEN PROCESSES SUPPORT MYELOMA CELL GROWTH AND SURVIVAL VIA DISTINCT REGULATORY AXES

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Background: We have previously reported that E2F1 and its heterodimerization partner DP1 promote MM tumor proliferation both in vitro and in vivo; and observed an inverse correlation between their expression and patient survival suggesting a role in MM pathogenesis. Moreover, E2F2 functional impairment by a dimerization inhibiting stapled peptide significantly affects myeloma tumor cell growth while sparing effect on normal components of bone marrows 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 ROSE2 (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 Pol II binding on H3K4me3 peaks. Distinct regulatory axes were defined for E2F1 and E2F2 functions. 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.

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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: 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 marrows 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 ROSE2 (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 Pol II binding on H3K4me3 peaks. Distinct regulatory axes were defined for E2F1 and E2F2 functions. 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.

Summary/Conclusions: In conclusions, our results highlight the existence of non-overlapping promoter and super-enhancer-associated dependencies in multiple myeloma, suggesting a sequestered molecular control that may be perturbed in cancer with potential for development of a promising therapeutic strategy.
Aims: We used a custom target pulldown (TPD) approach on a large cohort of MM 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, NRAS, TP53, FAM46C, BRAF, DIS3, TRAF3, SP140, IRF4) was found in 64%, with a long tail of infrequently mutated genes with uncertain significance. Karyotypic class was assigned in 80% of patients, with 9% of hyperdiploid cases also showing an IGH-Tx (mostly t(4;14)). IGH-Tx and aneuploidies dominated the MM genomic landscape, KRAS and NRAS being the only point mutations present in the 15 most frequent driver events. Multivariate analysis by separate Cox regression highlighted only four driver events with significant prognostic impact for both progression-free (PFS) and overall survival (OS): t(4;14) (HR 1.88, CI 1.25-1.84), amp(1q) (HR2.63, CI 1.92-3.59), del(17p) (HR2.53, CI 1.66-3.92), and rare mutations of ATP13A4 (HR 0.08, CI 0.01-0.65, mutated in 1.4% of patients). We found a significantly worse prognosis for increasing numbers of driver lesions in each patient (median OS 8.2 vs 3.5 years for <5 and>8 driver events, respectively). This was only partially explained by instances of additive effect or interactions between variables, which were very informative but not frequent. To better investigate these findings in the context of the genomic landscape of each case, we applied Bayesian clustering algorithms. The large number of driver events screened led to the identification of three groups: in the largest one, some hyperdiploid and IGH-Tx cases clustered together, suggesting that secondary mutations and CNAs required for tumor progression are often shared between these two subgroups. We then identified two clusters both characterized by significantly lower number of mutations, but with opposing features. One was enriched for IGH-Tx, had the highest number of CNAs overall, showed higher prevalence of amp(1q), del(13), del(17p), TP53 mutations, and had a shorter median OS of 5.3 years. The other was mostly composed of hyperdiploid cases and showed fewest CNAs and mutations, with a good prognosis (median OS not reached).

Figure 1.

Summary/Conclusions: We report on the first attempt towards the use of extended tumor genotype for a genomic classification of MM using innovative clustering algorithms. Despite the heterogeneity of the disease, we could identify disease subgroups with a distinct spectrum and number of driver events carrying different prognosis, supporting the introduction of genomics in the clinical approach to MM

E1202

A NOVEL METHOD FOR GENOME-WIDE COPY NUMBER ASSESSMENT FROM TARGETED SEQUENCING DATA AND CLINICAL APPLICATION IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Assessment of gene mutations by next generation sequencing is now standard in patients with haematological malignancy. However, larger chromosomal aberrations (e.g. exon, gene and chromosome level gains and losses) also serve as critical prognostic indicators that guide therapeutic decision making. These larger gene lesions are typically assessed using a separate methodology such as conventional cytogenetics/FISH.

Aims: We aimed to develop and clinically validate a novel method for assessing genome-wide copy number changes using an existing hybridisation-based targeted sequencing panel in order to provide further critical prognostic information not available from the existing targeted short read sequencing data without the need for a separate assay.

Methods: A custom Agilent SureSelect capture panel targeting 313 genes of relevance in myeloid and lymphoid malignancies was sequenced on an Illumina NextSeq (paired end 75bp reads) to a mean depth of 700x. An in-house bioinformatics pipeline was created to analyse probe counts from on-target and off-target regions, with which also detect CNAs and CNAs assessed for copy-number change and normalisation to a pooled reference comprising 10 normal controls. Three metrics for copy number calling were tested including a permutation-based statistic from circular binary segmentation, weighted mean and variance for the bins in each segmented region, and an MLPA-like test using ratios compared to controls. An interactive web-based graphical user interface was developed to visualise both large-scale and exon level amplification and deletions.

Results: We validated the approach on 45 samples from patients with multiple myeloma (predominantly advanced disease) with known copy number status as determined by conventional cytogenetics, FISH and MLPA. Our novel method detected numerous copy number changes that were outside the targeted region (through genome-wide mapping and analysis of off-target reads) such as del(1p) in 12 patients, gain(1q) in 15 patients and MYC amplification in 5 patients. Moreover our method was able to interrogate and resolve the complexity of changes on del(1p) including isolated deletions of FAM46C, CDKN2C and FAP1. Of 25 patients with a TP53 mutation, 20 had concomitant del(17p) detected by our assay, while 1 case had a del(17p) without mutation; both monosomic and biallelic TP53 aberration was associated with poor survival. Other findings in this cohort include frequent DIS3 mutations in patients with high-risk cytogenetics and 13 and 22q CNAs with monosomy 13 and hyperdiploid copy number changes such as the high amplification of KRAS 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 aberrations for patients with haematological malignancy. In the context of myeloma this can be used to report clinically relevant changes including deletions of 1p and 17p, and gains of 1q and 8q, as well as novel numerical chromosome aberrations.

E1203 THE MULTIPLE MYELOMA GENOME PROJECT: DEVELOPMENT OF A MOLECULAR 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 prognosis for patients. Current technologies have elucidated major translocation groups and recurrent copy number changes with varying effects on prognosis. However, minor translocation and mutation subgroups 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 develop a molecular segmentation strategy for MM to inform development and deployment of clinically relevant tests that could improve diagnosis, prognosis, and treatment of patients with MM.

Methods: We have established a dataset representing 1766 MM patients for which whole exome sequencing (WES; n=1367), Whole Genome Sequencing (WGS; n=474), and expression data from RNAseq and CNAs data sets (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 statistical methods.

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
80% power to detect gene expression changes and genomic variants associated in >2% of the study population. WES data identified the main cytogenetic groups, somatic variants, and significantly mutated genes. 28 significantly mutated genes were present in newly diagnosed samples (17 genes in >2% of samples). The main recurrent mutations included KRAS and NRAS, and negative regulators of the NF-kB pathway; however, novel genes were also identified. One distinct mutational patterns, proportions, and sites between translocation subgroups were found and will be presented. In addition, we detected recurrent copy number abnormalities and examined the interaction with mutations and fusion gene expression from RNASeq. Preliminary analysis with an integrative model developed with machine learning methods/approaches using CN, SNP, and structural variants predicted a subset of high-risk patients. Unsupervised molecular classification is in progress to integrate genomic data and define subgroups, which will be presented.

Summary/Conclusions: We have established the largest repository of molecular profiling data in MM associated with clinical outcomes. Integrated analyses are enabling generation of clinically meaningful disease segments associated with differing risk that will inform development of clinical tests. THEMp3 intends to build a global network by expanding collaboration with global MM centers to incorporate additional datasets through current and new collaborations.

*The first 6 authors share co-first authorship. The last 3 authors share co-senior authorship.

E1204

ALVOCIDIB SYNERGIIZES WITH VENETOCLAX IN PRECLINICAL MODELS OF MULTIPLE MYELOMA

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Background: With over 30,000 new cases expected in 2016 (US), new treatments are desperately needed for the treatment of multiple myeloma (MM). Major developments in the treatment of MM have included introduction of agents such as lenalidomide, thalidomide, or bortezomib. Bortezomib, an inhibitor of the proteasome, reduces the degradation of many proteins, including the pro-apoptotic protein NOXA. However, intrinsinc resistance to venetoclax treatment observed in MM patient samples has been attributed to a low BCL-2-to-MCL-1 gene expression ratio, suggesting a central role for MCL-1 in cell survival in this context as well. NOXA functions to sequester the anti-apoptotic BCL-2 family member, MCL-1. Increased MCL-1 expression is a known resistance mechanism to venetoclax treatment in a variety of cell types including chronic lymphocytic leukemia and lymphomas. Considering the central role of MCL-1 to treatment efficacy in MM, we investigated the ability of an MCL-1-lowering agent, namely the CDK9 inhibitor alvocidib, to potentiate the activity of venetoclax in MM. Alvocidib suppresses MCL-1 expression via CDK9-mediated repression and inhibited the tumor growth and inhibited the tumor growth in vivo, we performed a mouse study in the OPM-2 xenograft model.

Methods:

1. CellTiter-Glo and Caspase-Glo were used for cell viability and apoptosis assays investigating alvocidib and venetoclax in cell lines. We performed RT-PCR to measure mRNA levels of MCL-1 and other genes following treatment.

2. RNASeq was performed on RNA from untreated and treated MM cell lines and primary MM cells purified from patients but not in PBM-Cs from healthy donors. OSSL_325096 treatment leads to accumulation of poly-ubiquitinated proteins, cleavage of caspase-3, and up-regulation of CHOP in MM cell lines (Figure 2), suggesting this compound induces caspase-mediated apoptosis and ER-stress in MM cells. OSSL_325096 has a chemical structure similar to several known VCP inhibitors. Therefore, to evaluate the role of VCP in MM cell lines, we next performed knockdown of VCP. Knock-down of VCP induced apoptosis in MM cell lines, accompanied with accumulation of poly-ubiquitinated protein.

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 PBM-Cs from healthy donors. OSSL_325096 treatment leads to accumulation of poly-ubiquitinated proteins, cleavage of caspase-3, and up-regulation of CHOP in MM cell lines (Figure 2), suggesting this compound induces caspase-mediated apoptosis and ER-stress in MM cells. OSSL_325096 has a chemical structure similar to several known VCP inhibitors. Therefore, to evaluate the role of VCP in MM cell lines, we next performed knockdown of VCP. Knock-down of VCP induced apoptosis in MM cell lines, accompanied with accumulation of poly-ubiquitinated protein.

Summary/Conclusions: We have established the largest repository of molecular profiling data in MM associated with clinical outcomes. Integrated analyses are enabling generation of clinically meaningful disease segments associated with differing risk that will inform development of clinical tests. THEMp3 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.

E1205

NOVEL COMPOUND, OSSL_325096, INDUCES APOPTOSIS IN MULTIPLE MYELOMA CELLS THROUGH VCP INHIBITION

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Background: VCP (p97) is an ER-associated protein that belongs to the AAA ATPase family. It has a variety of cellular functions including ER-associated protein degradation, autophagy, and aggresome formation. Recent studies have elucidated emerging roles of VCP and its potential as a therapeutic target in several cancer subtypes including multiple myeloma (MM).

Aims: We screened approximately 2,000 small molecular compounds to find out novel small compounds that suppress growth of MM cell lines, and found that OSSL_325096 has strong anti-proliferative activity on MM cell lines (IC50 100-500µM). In this study, we evaluated anti-MM activity of OSSL_325096 through VCP inhibition, in an ATP-competitive manner.

Methods: OSSL_325096 were purchased from Princeton BioMolecular Research, Inc. (Princeton, NJ, USA). His-tagged human VCP (hVCP) cDNA was cloned and utilized to generate hVCP protein in vitro as previously described (Chou et al., PNAS, 2011, vol. 108(12): 4834-4839) to evaluate the VCP inhibition by OSSL_325096. For in 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 PBM-Cs from healthy donors. OSSL_325096 treatment leads to accumulation of poly-ubiquitinated proteins, cleavage of caspase-3, and up-regulation of CHOP in MM cell lines (Figure 2), suggesting this compound induces caspase-mediated apoptosis and ER-stress in MM cells. OSSL_325096 has a chemical structure similar to several known VCP inhibitors. Therefore, to evaluate the role of VCP in MM cell lines, we next performed knockdown of VCP. Knock-down of VCP induced apoptosis in MM cell lines, accompanied with accumulation of poly-ubiquitinated protein.

Summary/Conclusions: We have established the largest repository of molecular profiling data in MM associated with clinical outcomes. Integrated analyses are enabling generation of clinically meaningful disease segments associated with differing risk that will inform development of clinical tests. THEMp3 intends to build a global network by expanding collaboration with global MM centers to incorporate additional datasets through current and new collaborations.

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E1206

A NOVEL PREDICTIVE MODEL COMBINING LINCRNAS AND PROTEIN CODING GENES IN MULTIPLE MYELOMA

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Background: Cytogenetic aberrations are enabling generation of clinically meaningful disease segments associated with differing risk that will inform development of clinical tests. ThemP3 intends to build a global network by expanding collaboration with global MM centers to incorporate additional datasets through current and new collaborations.

Summary/Conclusions: The present data suggest that OSSL_325096 may be novel anti-myeloma drug candidate partially through its direct inhibition activity of VCP.

Madrid, Spain, June 22 - 25, 2017

haematologica | 2017; 102(s2) | 495
Background: RNA has diverse sets of regulatory functions and a recent analysis of the 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. lincRNAs have been considered to provide regulatory functions, however, their precise function and how they impact the patients remains unclear.

Aims: Here, we have studied lincRNAs using uniformly treated patients to show their impact on survival outcome in MM.

Methods: We performed RNA-seq on CD138+ MM cells from 360 newly-diagnosed patients and 18 normal plasma cells (NPM) and analyzed for lincRNA and protein-coding gene expression. The lincRNA data were analyzed using RNAseq analysis software.

Results: Using only the expressed lincRNAs, we developed a risk prediction signature with a AUROC of 0.57. We hypothesized that the expression of lincRNAs was associated with clinical outcome in MM patients and 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 risk prediction with other risk features improve the prediction power and helps to create better classification in MM.

E1207

DYNAMIC IMMUNOHISTOCHEMICAL EVALUATION OF MARROW MICROENVIRONMENT MODIFICATIONS IN PATIENTS WITH SMOLDERING MYELOMA

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Background: In most cases, multiple myeloma (MM) is preceded by an asymptomatic status known as monoclonal gammapathy 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.

Aims: Our work is focused on 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).

Results: Using only the expressed lincRNAs, we developed a risk prediction signature with an AUROC of 0.57. We hypothesized that the expression of lincRNAs was associated with clinical outcome in MM patients and 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 risk prediction with other risk features improve the prediction power and helps to create better classification in MM.

E1208

IMMUNE CELL PROFILING IN BONE MARROW OF MYELOMA PATIENTS POST AUTOLOGOUS STEM CELL TRANSPLANT SHOWS PRESENCE OF CYTOTOXIC CD4 AND CD8 CELLS, WITH PROMINENT LAG-3 EXPRESSION AND OTHER CHECKPOINT MARKERS

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Background: Multiple myeloma (MM) is a plasma cell malignancy that remains incurable, despite therapeutic advances. Immunotherapies have recently shown much promise in this and other cancers, and are under intense investigation. Autologous stem cell transplantation (ASCT) is standard of care in young fit newly diagnosed patients. In the post-ASCT setting, the minimal disease burden and re-constituting immune system may be a favourable context for immunotherapies, including cellular therapy and checkpoint blockade. Also, there is a need to characterize the post-ASCT BM, with a focus on identifying circulating T cells (BM) of MM patients post-ASCT, to identify candidate immune checkpoint proteins for therapeutic targeting.

Methods: BM aspirates were obtained from patients with MM at 3 months post ASCCT (n=28), and 6-12months post ASCCT (n=41) at University College Hosptial. Control BM aspirates were collected from healthy volunteers undergoing BM harvesting with the Anthony Nolan. Immunofluorescence surface staining was performed using antibodies to CD3, CD4, CD8, LAG-3, PD-1, HLA-DR, ICOS and the intracellular markers GzmB and Foxp3. All p-values indicate differences from normal donors unless otherwise stated.

Results: We observed the post-ASCT BM to be significantly higher numbers of cells exceeded CD3 cells as early as 3 months post-ASCT, suggesting the BM compartment is rapidly filled with CD8 cells. Although absolute numbers of CD4 effector cells (CD4+Foxp3-) were either similar (3 months) to or lower (6-12months, p<0.05) than healthy donors, there was a higher proportion of cytotoxic (GzmB+) CD3 cells (3months median 30.4%, range 0.2-89.7%, p<0.01 vs control median 2.6%, range 0.2- 33.4% and 6-12months, median17.6%, range 0.4-100%, p<0.05). CD4 effector cells also expressed activation markers: Inducible co-stimulator (ICOS, 3months median 20.2%, range 2.9-80.6% p<0.05 vs control median 8.6%, range 2.2-24% and 6-12months median 33%, range 1.8-80.7%, p<0.01) and HLA-DR (p<0.05), high levels of lymphocyte activation gene 3 (LAG-3) median 29.6%, range 12.2-62.1%, p<0.05 vs control median 17.9%, range 8.2-32.4%)

Significant numbers of CD4 effector cells co-expressed LAG-3 and GzmB (p<0.05), as well as LAG-3 and ICOS (p<0.05). Absolute numbers of CD8 effector cells (CD8+Foxp3-) in post ASCCT patients were not different from healthy donors.

Conclusion: In post-ASCT BM, significantly higher proportion of CD8 effector cells displayed cytotoxic phenotype (GzmB+), 3months median 88%, range 15.2-98.3%, p <0.0001 vs control median 30.3%, range 5.8-71.4% and 6-12months median 81.5%, range 19.4-100%, p <0.0001) as well as increased levels of HLA-DR (p<0.05) post ASCCT. CD8 effector cells also displayed high co-expression of LAG-3 and GzmB (p<0.05), but overall levels of LAG-3 were not significantly higher than controls. Both CD4 and CD8 effector cells expressed PD-1, however, levels were not significantly higher compared to healthy donors. Patients whose BM contained higher levels of activated (and cytotoxic) CD8 effector cells also had similar phenotype of CD8 effector cells. We correlated immune checkpoint protein expression in patients at 12months post ASCT. Patients with residual disease (PR) had higher levels of cytotoxic (Foxp3-GzmB+CD8) CD4 and CD8 effector cells (p<0.05) with co-expression of LAG-3 on cytotoxic effector cells, but no differences in PD-1 expression were seen.

Summary/Conclusions: The BM of MM patients following ASCCT shows activated CD4 and CD8 effector cells, 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.

E1209

INHIBITION OF EXTRACELLULAR VESICLE SECRETION INDUCES APOPTOSIS OF BONE MARROW STEM CELLS: TOWARDS SOIL-TARGETED THERAPY IN MULTIPLE MYELOMA

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122 nd Congress of the European Hematology Association
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 crosstalk is mediated by extracellular vesicles (EVs) plays an important role in the MM microenvironment.

Aims: In this study, we investigated the biological property of EVs and miRNAs in EVs derived from BMSCs, aiming to establish the emerging strategies to target MM microenvironment to prevent tumor growth and spread.

Methods: Bone marrow samples were obtained from MM patients (age 56 to 82, n=20) and monoclonal gammopathy of undetermined significance (MGUS) patients (age 44 to 82, n=13) in accordance with the Declaration of Helsinki and using protocols approved by the research Ethics Committee of Tokyo Medical University (IRB No. 2648), and BMSCs derived form MM patients (MM-BMSCs) and MGUS-BMSCs were isolated by the classical adhesion method. EVs were isolated from conditioned medium of BMSCs using a Exoquick-TC (SBI). The size of EVs was confirmed using a NanoSight LM10 (Malvern). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI).

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Aims: In this study, we investigated the biological property of EVs and miRNAs in EVs derived from BMSCs, aiming to establish the emerging strategies to target MM microenvironment to prevent tumor growth and spread.

Methods: Bone marrow samples were obtained from MM patients (age 56 to 82, n=20) and monoclonal gammopathy of undetermined significance (MGUS) patients (age 44 to 82, n=13) in accordance with the Declaration of Helsinki and using protocols approved by the research Ethics Committee of Tokyo Medical University (IRB No. 2648), and BMSCs derived from MM patients (MM-BMSCs) and MGUS-BMSCs were isolated by the classical adhesion method. EVs were isolated from conditioned medium of BMSCs using a Exoquick-TC (SBI). The size of EVs was confirmed using a NanoSight LM10 (Malvern). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI). The RNA from cells and EVs was profiled for 381 miRNAs using a TaqMan low-density array (ABI).
Results: Here we report that high expression levels of both G9a and GLP are associated with a worse disease outcome in 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-κB canonical pathway, IRF4 multiple myeloma program and mRNA splicing. Next, we screened specific G9a/GLP inhibitors BIX01294 and UNC1998 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 further indicated that BIX01294 treatment results in a shift of the G9a/GLP program 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 5TG1 inoculated mice with BIX01294 resulted in a clear antitumor response, as evidenced by a clear decrease in tumor burden and a significant increase in the overall survival of BIX01294 treated mice compared to vehicle treated mice.

Summary/Conclusions: Altogether, our results demonstrate for the first time the importance of the histone methyltransferases G9a/GLP in MM pathogenesis. Further, our data indicate that targeting G9a/GLP represents a promising strategy to improve treatment of MM.

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 immunosuppressive cytokines, notably the PD-1/PDL1 axis. Multiple myeloma is a disease characterised by a pre malignant phase which can evolve into periods of asymptomatic and symptomatic disease. One possible mechanism for disease progression is progressive loss of immunological control. The malignant plasma cell has multiple potentially immune modifying effects including the expression of PDL1 and induction of a pro-tumour micro-environment. The role of CTLs is less well understood.

Aims: To undertake deep immune profiling of the CTL landscape in myeloma in order to establish whether features of immune dysregulation are present and to identify potential therapeutic opportunities.

Methods: Cryopreserved bone marrow from 16 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. Dimensionality reducing clustering algorithmic analysis was used alongside traditional data analysis techniques to identify functional subpopulations characterised by expression of multiple markers.

Results: The cytolytic profile in newly diagnosed myeloma is shifted towards a pro-tumour microenvironment with particularly marked elevation of TGFb throughout resting CTLs (36.4% v. 66.2%, p<0.0001). IFNg production is reduced in the resting myeloma effector population (0.33% v. 0.18%, p=0.0099). Stimulation restores the cytolytic profile to match that of controls. Myeloma CTLs retain the capacity to proliferate and produce the constituents for cytotoxic granule formation, however elevated PD1 expression alongside other markers of exhaustion such as NKG2A and a transition towards the exhausted phenotype is occurring. Strongly PD1 expressing populations in myeloma are larger (26% v.43%, p=0.05) and have increased rates of co-expression of CTLA4 (32%, 64%, p=0.0015), PD1L (26% v. 47%, p=0.0198) and TIM3 (34% v. 56%, p=0.0241). Populations of CTLs from myeloma up-regulate expression of the TCR co-stimulatory molecule CD28 (74% total CD3, NKGD2 (45% total CD8) and OX40 (33% total CD8) following stimulation.

Summary/Conclusions: Clear differences can be identified in the functional and phenotypic features of CTLs in myeloma compared to those of controls. The partial nature of these defects and the fact that reversibility can be demonstrated suggest that these cells have not yet reached the stage of irreversible exhaustion. Taken together this data suggests that targeting immune checkpoint regulators at an early disease stage, in order to optimise immunological function and reverse partial defects, is a viable therapeutic strategy to explore. PD1, PD1L, CTLA4 and TIM are all potential immune checkpoint targets. In addition the expression of specific G9a/GLP inhibitors BIX01294 and UNC1998 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 further indicated that BIX01294 treatment results in a shift of the G9a/GLP program 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 5TG1 inoculated mice with BIX01294 resulted in a clear antitumor response, as evidenced by a clear decrease in tumor burden and a significant increase in the overall survival of BIX01294 treated mice compared to vehicle treated mice.

Summary/Conclusions: Altogether, our results demonstrate for the first time the importance of the histone methyltransferases G9a/GLP in MM pathogenesis. Further, our data indicate that targeting G9a/GLP represents a promising strategy to improve treatment of MM.

E1213
P53-RESTORING SMALL MOLECULE CP-31398 INDUCES APOPTOSIS VIA INDUCTION OF REACTIVE OXIDATIVE SPECIES IN HUMAN MULTIPLE MYELOMA
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Background: Reactive oxygen species (ROS) are normal byproducts of a wide variety of cellular processes. ROS have dual functional roles in cancer cell pathophysiology. At low to moderate levels, ROS act as signal transducers to activate cell proliferation, migration, invasion, and angiogenesis. In contrast, high levels of ROS induce cell death. In multiple myeloma (MM), ROS overproduction is the trigger for apoptosis induced by several anticancer compounds, including proteasome inhibitors. However, no drugs that mainly affect oxidative stress are currently used for treatment of MM in the clinic. In MM, p53 status is an independent prognostic marker, since patients harboring p53 abnormalities are highly resistant to standard therapies, and the incidence of p53 mutations and deletions increases during disease progression. Therefore, restoration of p53 is an attractive strategy for treating advanced relapsed and refractory MM (RRMM) patients. CP-31398 (CP) is a small molecule that activates wild-type p53 or restores tumor-associated p53 mutants to wild type p53 function in multiple human cancer cell lines; this leads to cell cycle arrest and/or apoptosis. The growth of thymomasomea cell lines can be inhibited by p53-dependent induction of ROS, but it is not clear whether CP-induced cytotoxicity proceeds via a similar pathway.

Aims: Our study was aimed at evaluating the anti-myeloma activity of CP.

Methods: MM cell lines (MM1S, RPMI8226, U266, KMS5, OPM2, Delta47, KMS11) and three primary treated for 48 h. Consequently, the inhibitory effect of CP on MM cell line growth was assessed using a WST-1 assay. In order to elucidate the cytotoxic mechanism of CP, immunoblotting and flow cytometry analysis were performed. Measurements of cytosolic and mitochondrial ROS were performed with CellROX Deep Red or MitoSOX Red. For quantification of ROS, cells were analyzed by flow cytometry and fluorescence 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 carfizomib (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 FROMmGUS TO MYELOMA IS ASSOCIATED WITH PRO-TUMORAL ACTIVATION OF MES-enchymal STROMAL CELLS (MSC)
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Background: A well-recognized feature of MM is the intimate relationship between plasma cells (PC) and bone marrow microenvironment, characterized by a modified extracellular matrix, enhanced angiogenesis and presence of cells with immune suppressive activity, including tumor-associated macrophages and myeloid-derived suppressor cells (MDSC). Recently, we demonstrated that MM-MSCs are able to convert normal immature myeloid cells in MDSC contributing to immune-escape mechanisms.

Aims: We hypothesize that MSC derived from Smoldering myeloma (SMM) and MM are in an activated status that promotes tumor growth and tumor microenvironment transformation.

Methods: Human peripheral blood mononuclear cells (PBMC) isolated from healthy controls (HC), myeloma (MM)-, MM-MSC- or MM-MSC- and were cultured with healthy controls (HC)-mgUS-, 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 angiogenesis and suppress T cell proliferation.
E1215
LONG TERM CR MULTIPLE MYELOMA PATIENTS STUDIED WITH NEXT GENERATION FLOW SHOW PREDOMINANTLY CURED VSMGUS-LIKE MINIMAL RESIDUAL DISEASE PATTERNS: A STUDY OF THE GTMM-TUSCAN GROUP FOR MULTIPLE MYELOMA
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Background: CR is a prerequisite for long term responses, progression free survivals, and ultimately overall survivals and cure. In the era of novel agents, many MM patients can achieve stringent CR (sCR), i.e. disease disappearance at serological, immunohistochemical level plus negativity of free light chains (FLC). On the other hand most of these patients still will relapse and minimal residual disease (MRD) detection will play a crucial role in the very next future. Recently, two  colours tubes panel developed by the EuroFlow Consortium can detect MRD with an increased sensitivity and can be applied as standardized method to study multiple myeloma (MM) patients.

Aims: While many studies have looked at MRD status sequentially and soon after autologous or allogeneic stem cell transplantation with flow or molecular techniques, little is known about long term remission patients (>5-10 years) and in particular if more sensitive techniques such as NGF or NGS can still detect minimal residual disease in those patients. Aim of the study was to analyse patients with MM in >VGPR with next generation flow at >2 and >5 years of lasting remission.

Methods: Clinical assessment definition of CR status included serum and urine immunofixation, free light chain determination, imaging study with CT-PET, bone biopsy. Bone biopsy was performed at core biopsy. In 50 MM patients (M/F=30/20), were studied with NGF at two GTMM centers between February 2016 and February 2017. 28/56 (50%) patients were in sCR at the moment of the study at a median of 40 months after therapy (range 3-140). 28/50 (50%) patients were in VGPR at study analysis according to new IMWG response criteria. N= 12, 25 and 44 patients had a remission disease >5 years, >2 years, and <5 years, respectively.

Two tube assay incorporated 8 antibodies each: CD38, CD56, C2M-Microglobulin, CD19, k Anti-Kappa Anti-Lambda CD45 DCD5, CD38, CD28, CD27, CD119, CD81, CD45 and CD138 (OneFlow™ PCST and PCD, BD Biosciences) and were utilized to detect MRD level with a lyse-wash-and-stain protocol driven by flow cytometry (FACScanto II, BD Biosciences). Accurate identification of BM plasma cells (PCs) and discrimination between phenotypically aberrant (pAPC) and normal PC (pNC) were carried out after acquisition and analysis of >2 × 10⁶ cells (Diva 8, BD Biosciences).

Results: MRD+ status was detected in 23/56 (41%) of the patients. 4/12 (33%) were MRD positive at >5 years remission (2 sCR, 2 VGPR) (median 96 months range 72 – 186 months); 20/44 (45%) were positive at >5 years of remission (3 CR; 17 VGPR)(median 9.5 range 3 – 46 months). 9/25 (36%) were MRD+ after >2 years of remission (2 sCR, 7 VGPR) (median 46 months range 24 – 186 months). As expected being in sCR was correlated with a low MRD+ prevalence (10%) in VGPR patients after >5 years. Interesting looking at long lasting remission, i.e. >5 years, the 4/14 patients that resulted MRD+ displayed anmgUS like –plasmacell immunophenotype (prevalence of normal plasmacells vs aberrant mononuclear) with a PCH/PCtot ratio of 48%, 95%, 35%, 30%. CTIPET was positive in 22/56 patients. All patients with negative MRD had a CR status.

Summary/Conclusions: In conclusion NGF showed that MM patients with long remission status can be considered disease free/cured with a high sensitivity method. MM patients that display anmgUS-like phenotype after achieving 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.

E1216
THE NOTCH PATHWAY IN THE INTERPLAY BETWEEN MYELOMA CELLS AND ENDOTHELIUM IN THE BONE MARROW NICH
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Background: Angiogenesis is a hallmark of tumors, and it is a peculiar characteristic in bone marrow (BM) of multiple myeloma (MM) patients. MM is a still incurable disease that strongly depends on interactions with BM microenvironment. Endothelium of MM patients displays malignant behavior as compared to a healthy counterpart (1). MM displays a dysregulation of the Notch pathway due to Jagged ligands and Notch receptors overexpression. This condition brings to the generation of homotypic and heterotypic interaction loops that sustain MM cells growth. However, Notch signaling is mainly involved in 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, Jagged1 and 2, were silenced in the MM cell line RPMI8226 (RPMI8226shJAG1/2) using an inducible lentiviral vector carrying two short hairpin RNAs targeting Jagged1 and 2. To mimic the endothelial compartment, primary endothelial cells (EC) and artificial endothelial cells (HPCAECs) were used and for the stromal compartment, the GFP+H55 cell line. Matrigel and scaffold healing assays were set up to investigate Notch role in modulating not only the angiogenic potential of MM cells co-cultured with HPCAECs and HPCAEC 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 HPCAECs by flow cytometry.

Results: Matrigel assay of HPCAEC co-cultured with MM cells showed that direct contact increased angiogenic potential of HPCAEC to form a grid of tubes; this effect is significantly reduced when HPCAECs are co-cultured with RPMI8226shJAG1/2 cells, indicating a key role of Notch signaling in endothelial stimulation. Wound healing assay demonstrated that Notch signaling affects EC motility; since it is reduced when Jagged 1/2 are silenced. 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 micromimics BM microenvironment and may be used as a novel tool to recapitulate the interactions of BM and tumor cells beyond the animal models.

References
**Aims:** To understand the role of stromal induced drug resistance and to identify new therapeutic targets in myeloma.

**Methods:** GFP-tagged human myeloma cell lines, 8226, U266 and MM.1s, were co-cultured with MM patient-derived BMSCs 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 BMSCs 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 survivin expression was monitored in BMSCs in presence or absence of BTZ. GFP fluorescence-based adhesion, cytotoxicity and annexin-V/PI apoptosis were applied.

**Results:** qPCR arrays showed that BMSCs up- or down-regulated several miRNAs and mRNAs in MM cells. Survivin (BIRC5) was confirmed to be consisently increased in the MM cell lines and mRNA and protein levels. In contrast, miR-101-3p was confirmed to be significantly downregulated by stroma in MM cells. Moreover, suppression of miR-101-3p or upregulation of survivin was reversed partially when BMSCs were pre-treated with BFA but highly significantly when they were separated from MM cells with a TW insert. The same regulatory network including western blotting and mRNA or miRNA qPCR arrays indicating that direct cell-direct cell adhesion was more effective in BMSC-induced modulations in MM cells. Next we identified that survivin was a direct target of miR-101-3p, overexpression of miR-101-3p suppressed survivin mRNA/protein. As indicator of involvement in stroma-mediated drug resistance, survivin and miRNA-101-3p expression in MM cells being BMSCs co-cultured was measured. Furthermore, miR-101-3p overexpression or silencing of survivin increased BTZ-induced apoptosis in MM cells in the absence or presence of BMSCs significantly overcoming stroma-mediated drug resistance. To test whether miR-101-3p could also regulate adhesion of MM cells to BMSCs, we assessed whether 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 BMSCs induce drug resistance in MM cells by upregulating survivin and downregulating miRNA-101-3p which directly targets survivin. Overexpression of miRNA-101-3p or silencing of survivin sensitizes MM cells to BTZ significantly overcoming stroma-induced drug resistance. These findings disclose a role of survivin-miRNA-101-3p axis in regulation of MM cell adhesion to BMSCs and thus provide a rationale to further investigate the anti-myeloma activity of miR-101-3p in combination with BTZ as a potential therapeutic strategy in MM.

**E1218**

**ARQ-197, A SMALL-MOLECULE INHIBITOR OF C-MET, REDUCES TUMOUR BURDEN AND PREVENTS TUMOUR-ASSOCIATED BONE DISEASE IN A MURINE MODEL OF MYELOMA**

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**Background:** The receptor tyrosine kinase c-Met, its ligand HGF, and their signaling pathway, have all been implicated in the pathogenesis of myeloma. In myeloma patients with elevated levels of HGF their prognosis is known to be worse.

**Aims:** To unravel the role of the bone marrow mesenchymal/stromal cells (BMMSCs) in MM cell growth, progression and drug resistance.

**Methods:** Hypothesizing that the interaction between MM cells and the BMMSCs is bidirectional, we have compared BMMSCs from healthy individuals, mouse 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, mouse, and MM patients, as well as BMMSCs impacted by MM in our humanized bone marrow-like model, allowed us to confirm established disease biomarkers (e.g. IL-...
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 BMMSC-induced growth of MM plasma cells and drug resistance, we have established HSTS stromal cell lines that undergo CRISP/Rcas9 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-18, CXCL8, CSF2 and CSF3). 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 BMMSCs, it certainly is not the sole contributor to BMMSC-induced MM cell growth and drug resistance.

**E1222**

**THE PAN-PIM KINASE INHIBITOR, PIM447, POTENTLY SYNERGESIZES 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 targets in MM. Recent work from our group has shown the dual antitumorogenic 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 Calcusyn software based on results from MTT assay. Effects on apoptosis and cell cycle were evaluated by flow cytometry. Glucose uptake was analyzed by incubation with 2-NBDG. The results from MTT assay. Effects on apoptosis and cell cycle arrest at G0-G1 phase. Accordingly, cleavage of caspase 3 and PARP, as well as reduction of cyclin D2 was observed by western blot. In addition, triple combination inhibited mTORC1 as shown by analysis of different protein levels by western blot. In addition, triple combination inhibited mTORC1 as shown by analysis of different protein levels by western blot.

**Results:** Triple combination PIM447 + pomalidomide + dexamethasone showed a strong synergism (CI<0.3) in all cell lines tested. The combination was able to induce apoptosis and cell cycle arrest at G0-G1 phase. Accordingly, cleavage of caspase 3 and PARP, as well as reduction of cyclin D2 was observed by western blot. In addition, triple combination inhibited mTORC1 as shown by analysis of different protein levels by western blot. In addition, triple combination inhibited mTORC1 as shown by analysis of different protein levels by western blot.

**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.

**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 (PIGOR) we identified aberrant expression of an E3 ligase TRIM33 (tripartite motif containing protein 33) in MM. TRIM33 has previously been identified as a tumor suppressor for myelomonocytic leukemia and hepatocellular carcinoma.

**Aims:** The aim of this study was to examine TRIM33 expression and to investigate its role as a potential tumor suppressor in MM.

**Methods:** Western blotting was used to analyse TRIM33 expression at basal level and following knockdown in four MM cell lines representing a range of MM translocations; JJN3 t(4;14), U266 t(11;14), KMS-18 t(4;14), OPM-2 t(4;14). TRIM33 knockdown was performed using shRNA pLKO lentiviral plasmids. CellTiter-Glo® was used to determine cell viability following knockdown. A range of MM translocations were used to examine 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 (4;14) cell lines, KMS-18 and OPM-2. Conversely, expression was found to be high in the non (4;14) cell lines, JJN3 (p=0.001) and U266 (p=0.015). Knockdown of TRIM33 expression did not alter cell viability in the (4;14) cell lines. However, cell viability was found to be increased in JJN3 (p=0.004) and U266 (p=0.005). Analysis of a publicly available dataset, GSE19784, showed lower levels of TRIM33 present in patients with a (4;14) compared to other MM subtypes, particularly (6;14) (p=0.004) and hyperdiploid cluster (p=0.03). Low TRIM33 expression has also been associated with poor overall survival (GSE2658; p=0.0034). Forty-seven myeloma patients were compared with TRIM33 expression in non (4;14) cell lines. Identification of an E3 ligase TRIM33 (tripartite motif containing protein 33) for MM was identified; these genes were analysed using QUADARTIC tools connectivity mapping to identify FDA approved agents predicted to enhance the TRIM33 gene signature.

**Conclusion:** The aim of this study was to examine TRIM33 expression and to investigate its role as a potential tumor suppressor in MM.
enhancer of the TRIM33 signature that potently decreased the viability of the OPN2 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 lncRNA transcriptome of MM and its heterogeneity, and determine whether altered lncRNAs have a functional involvement in this disease.

Methods: Paired-end strand-specific RNA sequencing (ssRNA-seq) was performed in 38 purified plasma cell (PC) samples from MM patients, as well as in 5 tonsil PCs (TPCs) and in 3 bone marrow PCs (BMPCs) of healthy donors as controls. We also performed ssRNA-seq of populations from B cell differentiation (Naïve, Germinal Center, Memory and PC). To study the heterogeneity of lncRNAs expression we performed sample level enrichment analysis (SLEA), in which each individual lncRNA was compared to BMPCs. To determine the epigenetic regulation of lncRNAs we used whole-genome bisulfite sequencing and CHIP-seq. shRNA-mediated knockdown using 2 different shRNAs and MTK-8 or Proliferin V (cell death) assays were utilized to study the functional effect of lncRNA overexpression.

Results: We identified 40.552 novel lncRNAs in MM samples that were present in at least 3 of the 38 patients. Principal component analysis demonstrated that TPCs and BMPCs cluster separately, suggesting that, in spite of being the same disease, their transcriptomes are very different. We observed that the expression of lncRNAs was more heterogeneous than that of coding genes. More importantly, SLEA showed 11.067 lncRNAs that were overexpressed and 5.601 underexpressed in >40% of patients. Thus, the number of deregulated genes analyzed by SLEA was much larger than the 75 lncRNAs 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 lncRNAs in the biology of MM cells we focused on the study of LINC-SMILO (Specific Myeloma iNergetic Long non-coding RNA), a lncRNA that it is overexpressed in ~40% of MM patients and not altered at the transcription level. LINC-SMILO demonstrated that CpGs located upstream of LINC-SMILO showed a significant hypomethylation in mmGlUS, that was even more pronounced in MM samples. These data suggest that epigenetic dysregulation of lncRNAs may play an important role in the biology of MM cells.

Conclusions: These data demonstrate that alteration of lncRNAs is an important and unexplored feature of MM. Moreover, overexpression of LINC-SMILO is required for the survival of MM cells and could represent a potential therapeutic target for the treatment of this disease.

E1226
ROLE OF EPHA3 IN MULTIPLE MYELOMA: A PERSPECTIVE FOR A NOVEL TARGET THERAPY?
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Background: The tyrosine kinase Eph receptor A3 (EphA3) has recently emerged as a potential therapeutic target, since it has been overexpressed in many cancers, including some hematological malignancies (Keane et al. 2012). Furthermore, EphA3 has been found overexpressed not only in neoplastic cells, but also in the microenvironment of different human cancers, where its targeting inhibits tumor growth by disrupting supportive stroma and vasculature (Vail et al. 2014).

Aims: Due to the absence of relevant information about the role of EphA3 in multiple myeloma (MM), we aimed to evaluate the expression of this molecule in primary bone marrow plasma cells (BMPCs) from MM patients and MM cell lines compared to healthy controls (HCs). In addition, using a “loss of function” approach by mRNA silencing and an anti-EphA3 monoclonal antibody (EphA3mAb), we studied in vitro plasma cells (PCs) viability and movement. Finally, we analysed the in vivo effects of EphA3mAb in a MM mouse xenograft model.

Methods: EphA3 mRNA and protein where investigated in 15 MM BMPCs, 11 MM cell lines and 10 HCs by qRT-PCR and flow cytometry. The effects of EphA3 targeting by lentiviral RNA silencing (shRNA) and anti-EphA3mAb on PC trafficking and viability were studied by adhesion assay on fibronectin and on bone marrow stromal cells (BMSCs), invasion assays and proliferation MTS assay, respectively. Gene expression profiling (GEPI) was performed in shEphA3 versus shControl cells. Furthermore, the effects of EphA3mAb were analysed in MM xenograft model by measuring tumor size and by assessing angiogenesis, proliferation and apoptosis rate on tumor biopsies using immunohistochemistry (anti-CD31, anti-ki67 and TUNEL assay, respectively). Statistical significance was determined by the t-test or One-way ANOVA analysis.

Results: EphA3 was found overexpressed in primary MM BMPCs and MM cell lines when compared with HCs (figure 1A-B). The EphA3 loss of function by siRNA and by EphA3mAb significantly inhibited in vitro the ability of MM PCs to adhere to fibronectin, to BMSCs and to invade (figure 1C-E), without affecting cell proliferation and viability (data not shown). GEPI showed that knockdown of EphA3 modulated some molecules that regulate adhesion, migration and invasion processes. Importantly, the treatment with EphA3mAb in vivo significantly reduced tumor size and inhibited angiogenesis, as revealed by decrease of CD31+ vessels at immunohistochemistry (data not shown).

Figure 1.
Summary/Conclusions: Our findings suggest that EphA3 is a novel regulator of MM PC trafficking, in part via effects on adhesion and invasion; its targeting using EphA3mAb inhibits tumor growth, possibly due to 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 1p32/1q21, XL IGH plus, XL t(11;14), XL t(4;14), XL t(14;16), XL t(14;20), XL t(6;14), XL cMYC BA, XL 5p15/9q22/15q22, XL P53 (MetaSystems), D13S25 (Cytocell). Induction therapy with bortezomib-based courses was initiated for 131 pts, 3 pts with smoldering MM remained under observation. Response was evaluated according to the IMWG criteria for 127 pts, 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. T(14q14/22q11) was detected in 42.5% (57/134), hyperdiploidy in 57.5% (77/134), hypodiploidy in 2.4% (3/134) pts. In 11.2% (15/134) a concurrent t(11g12) 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(13)(q) was detected in 40.3% (54/134), del(17)(p) in 12.7% (17/134), t(4;14)(q21;q2), t(14;22) in 17.2% (23/134). Amp1q21 was detected in 39.6% (53/134). We identified 3 copies of 1q21 in 54.3% (73/134), del(13)(q) in 36.3% (49/134), t(14;22) in 19.3% (26/134), t(4;14) in 16.5% (22/134), del(17)(p) in 37.5% (50/134). In 9 pts (6.7%) we detected 4 copies of 1q21. Amp1q21 was detected also in disease progression.


Figure 1.

Summary/Conclusions: Our results show that amp1q21 has a significant impact on OS MM pts in cases of more than 3 copies of locus only. In cases of 3 copies of 1q21 OS pts is comparable with OS in group without amp1q21. Amp1q21 can appear in the course of MM, therefore FISH-analysis of locus 1q21 should be performed at diagnosis, as well as in disease progression.
Methods: Human BM-MNCs derived from normal human subjects or MM patients were cultured with M-CSF plus rRANKL with or without 50µM of K262Ab for OC formation for TRAP staining and functional assay. To assess the mechan-isms of action of CD26Ab on OCs, RANK signaling proteins were examined by immunoblotting.

Results: CD26 is expressed on normal human Ocs and is intensely expressed on activated Ocs in MM, MGUS, and MM cell lines (MANK) induced human OC differentia-tion, in association with CD26 expression on monocyte-macrophage lineage cells. CD26 expression was accompanied by increased phosphorylation of MK2/3 and p83MAPK, which is crucial for human OC differentiation with its downstream activation of microphthalmia-associated transcription factor (mTOR) plays an important role in OC function. CD26 expression was associated with the number of multinucleated Ocs (3>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 CD26mAb, which is a known inhibitor of OC precursor cells (MANK/3p58MAPK-mi/Mitf), was not phosphorylated at all in immature Ocs. After RANKL stimulation, regardless of the absence or presence of CD26mAb. These results suggest that CD26mAb blocked RANKL induced p83MAPK phosphorylation in OC precursor cells, but not in OCs. The activation of other MAPKs including ERK and SAPK/JNK, or NFκB was rapidly induced in response to RANKL both in OC precursor cells and in mature OCs. The absence of phosphorylation of CD26mAb did not directly affect mature OC functions. Next, although CD26mAb 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, co-cultures of Ocs and MM cells, the percentage of expression of CD26mAb did not directly affect mature OC functions. Our data imply that the blockade of CD26 signaling with CD26mAb impairs the development of human functional Ocs. Targeting CD26 in both Ocs and MM cells with CD26mAb may be a promising novel therapeutic strategy in MM-associated bone disease and MM progression.

E1230
KYNURENINE INHIBITS T-CELLS THROUGH THE ARYL HYDROCARBON RECEPTOR AT IDO-POSITIVE TUMOR MICROENVIRONMENT
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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 impor-tance 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 of PERK, while displaying ≥385 fold selectivity over c-Kit, aurora B, BRK and many other kinases.

Aims: In this study we aimed to use a small ATP competitive molecular inhibitor, GSK2606414, that acts by targeting PERK enzyme activity in its inactive DFG conformation at the ATP-binding region, while displaying ≥385 fold selectivity over c-Kit, aurora B, BRK and many other kinases.

Methods: We initially screened 25 CD138+ MM patients and 6 human myelo-ma cell lines (HMCls) for PERK mRNA expression. Our results showed that PERK mRNA is highly expressed in almost all patients (5-10 fold higher than the mean PERK expression of HMCls).

Results: We tested 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 GSK2606414 for 24 hours resulted 25% and 15% increase in apoptotic cells by Annexin-V/PI staining respectively compared to the untreated cells. However, the most important finding was a significant synergistic effect of GSK2606414 with bortezomib in these cells. Specifically when H929 and L363 cells were treated with 5nM bortezomib in combination with 20µM GSK2606414, synergistic effect was seen where apoptotic cells reached 99% and 77% respectively, compared to bortezomib-treated cells (28% and 42% respectively). In addition, the effect of GSK2606414 in combination with bortezomib in the proliferation of H929 and L363 cells was examined. As seen in the apoptosis assay, pre-treatment with GSK2606414 for 24 hours caused in 40% and 30% reduced cell proliferation in H929 and L363 respectively compared to bortezomib only treated cells. Under ER stress conditions, the activation of ATF6 and PERK/eIF2α leads to the induction of ATF4 translation and results in the upregulation of CHOP. To determine the gene target effects of GSK2606414, ATF4 and CHOP mRNA expression levels were determined in H929 cell line after 24 hour of treatment. Treatment with GSK2606414 alone did not alter the expression levels of CHOP but reduced more than 50% the expression levels of ATF4. When combined with bortezomib CHOP and ATF4 levels were reduced 20% and 60% respectively while treatment with bortezomib alone reduced CHOP and ATF4 expression levels of 40% and 30% respectively. Changes in RNA expression of 84 UPR-related genes were analyzed in H929 cells. Specifically H929 cells were pre-treated with GSK2606414 and then subjected to ER stress conditions by treatment with tunicamycin (TM). After 24 hours of treatment, 50 genes were found to be transcriptionally regulated by 5-fold in response to TM- stress conditions. The top 10 genes (EERP1, ENRD, XBP1, DDIT3, CEBPB, PPP1R15A, etc.) were downregulated by ≥5 fold, whereas 10 of these genes (HERPUD1, E1F2AK3, CREB3L3, HSP2A, HSPA1B, etc.) were upreg-ulated similarly.
**Summary/Conclusions:** In conclusion, given the on-target pharmacological effects of PERK inhibitor on MM, development of PERK inhibitors may offer a therapeutic advantage that would affect MM pathogenesis and treatment.

**E1232**

**ENVIRONMENTAL CONTROL OF PLASMA CELL FITNESS IN MULTIPLE MYELOMA: MALIGNANT CO-OPTATION OF ARGinine AS NOVEL IMMUNOTHERAPEUTIC STRATEGY**

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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 autophagy activity that is highly dependent on the PC fitness sustaining p62 expression. To investigate this hypothesis, in this study we defined a novel BM niche and its impact on MM PC fitness.

**Aims:** We hypothesized that in MM BM niche sustain PC fitness by maintaining p62 expression.

**Methods:** We integrated diverse unbiased and hypothesis-driven approaches: (1) gene expression profiles (GEP) of patient-derived circulating HDNs (60 MM, 30 healthy, 30 controls); (2) metabolic profiling by UHPLC/MS/MS of ad-hoc collected BM and peripheral plasma (16 MM, 17 smoldering MM, 30 healthy, 29 controls), and (3) functional and expression analysis in vitro studies on human MM cell lines. We validated our observations in primary MM cells using bioinformatic analysis of transcript expression levels detected by RNAseq.

**Results:** Gene expression analysis demonstrated that ISS, revised ISS (R-ISS), abnormal LDH, plasma myoglobin, and p62 expression are higher in 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 with ISS3 and CKD-EPI <40ml/min/1.73m2 and 3% of patients with ISS1 and CKD-EPI ≥40ml/min/1.73m2 (p = 0.001). The incidence of EM in each group was 8.1% 39% and 15.3%, respectively (OR: 2.8, 95% CI: 1.9-4.1, p < 0.001). Multivariate Cox regression analysis of prognostic factors for OS in the whole population demonstrated that CKD-EPI/ISS model was the strongest independent prognostic factor for OS (HR= 0.38, 95% CI: 0.28-0.50, p < 0.001).

**Summary/Conclusions:** Based on our data, the combination of eGFR estimated by CKD-EPI with ISS (CKD-EPI/ISS) represents a powerful independent prognostic model for MM and OS, in the era of novel agents. The markers constituting ISS and eGFR are cheap and available for most of MM patients, therefore the CKD-EPI/ISS prognostic model is applicable. Nevertheless, the establishment of CKD-EPI/ISS model requires further validation.

**E1233**

**ESTIMATED GLOMERULAR FILTRATION RATE (eGFR) CALCULATED BY CKD-EPI EQUATION COMBINED WITH THE INTERNATIONAL STAGING SYSTEM PROVIDES A POWERFUL PROGNOSTIC MODEL FOR EARLY MORTALITY IN MYELOMA PATIENTS**

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**Background:** Among putative pro-tumoral players are immunosuppressive BM-derived high-density neutrophils (HDNs), but their role in MM is unknown.

**Aims:** We hypothesized that in MM HDNs sustain PC fitness sustaining p62 expression through 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 controls), (2) metabolic profiling by UHPLC/MS/MS of ad-hoc collected BM and peripheral plasma (16 MM, 17 smoldering MM, 30 healthy, 29 controls), and (3) functional and expression analysis in vitro studies on human MM cell lines. We validated our observations in primary MM cells using bioinformatic analysis of transcript expression levels detected by RNAseq.

**Results:** The incidence of EM was higher in MM group compared with the rest of the patients (69% vs 17%, p < 0.001). The causes of EM included infection/sepsis: 54%, high risk cytogenetics including t(4;14), t(14;16), and del17p were present in 48% of patients with ISS3 and CKD-EPI <40ml/min/1.73m2 and 3% of patients with ISS1 and CKD-EPI ≥40ml/min/1.73m2 (p = 0.001). 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 ISS1 and CKD-EPI ≥40ml/min/1.73m2, 2) high risk group including patients ISS3 and CKD-EPI <40ml/min/1.73m2 and 3) intermediate risk group that did not fit in either low or high risk groups. The incidence of EM in each group was 8.1% 39% and 15.3%, respectively (OR: 2.8, 95% CI: 1.9-4.1, p < 0.001). Multivariate Cox regression analysis of prognostic factors for OS in the whole population demonstrated that CKD-EPI/ISS model was the strongest independent prognostic factor for OS (HR= 0.38, 95% CI: 0.28-0.50, p < 0.001).

**Summary/Conclusions:** Based on our data, the combination of eGFR estimated by CKD-EPI with ISS (CKD-EPI/ISS) represents a powerful independent prognostic model for EM and OS, in the era of novel agents. The markers constituting ISS and eGFR are cheap and available for most of MM patients, therefore the CKD-EPI/ISS prognostic model is applicable. Nevertheless, 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 CLONOGENIC 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 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 isolated and cocultured in the genetically modified K562-mb15-4T1BL cells in order to obtain NKAES. The activity of NKAES cells against SP was evaluated by time lapse microscopy and the activity against CTCs was evaluated by methylcellulose assay. In vitro safety against CD34 + progenitors was evaluated by time-resolved fluorescence cytotoxicity with europium-TDA and cul- turing of HMC-1, BCP-1 or lymphoblastoid cell lines with CD34 + progenitors. SP cells from both cell lines and samples from different stages of MM showed overexpression of stemness markers. Patient NKAES cells were shown to have much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES cells were evaluated to detect and co-cultured in the genetically modified K562-mb15-4T1BL cells in order to obtain NKAES cells. The activity of NKAES cells against SP was evaluated by time lapse microscopy and the activity against CTCs was evaluated by methylcellulose assay. In vitro safety against CD34 + progenitors was evaluated by time-resolved fluorescence cytotoxicity with europium-TDA and culturing of HMC-1, BCP-1 or lymphoblastoid cell lines with CD34 + progenitors.

**Results:** SP cells from both cell lines and samples from different stages of MM showed overexpression of stemness markers. Patient NKAES cells were shown to have much higher adhesion, migration and detection capacity of MM tumor cells than NK cells from the same patient. After SP purification by sorting, NKAES cells were evaluated to detect and co-cultured in the genetically modified K562-mb15-4T1BL cells in order to obtain NKAES cells. The activity of NKAES cells against SP was evaluated by time lapse microscopy and the activity against CTCs was evaluated by methylcellulose assay. In vitro safety against CD34 + progenitors was evaluated by time-resolved fluorescence cytotoxicity with europium-TDA and culturing of HMC-1, BCP-1 or lymphoblastoid cell lines with CD34 + progenitors.
UNMASKING THE RETROTRANSPOSEN-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 increased 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 overexpression in primary MM cells and human MM cell lines has been validated in three independent studies. Furthermore it was recently demonstrated that sRANKL mRNA promoter and first exon are of retropositional origin, residing within a large genomic cluster of transposable elements (TEs).

Aims: To unmask the TE-shaped transcriptional and epigenetic apparatus impelling the expression of sRANKL mRNA in a cell-type and cell-context-specific manner.

Methods: RepeatMasker software was used to reveal the presence of integrated TEs in the genomic segment comprising TNFSF11. 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 Methy450 ENCODE track.

Results: RNA-Seq data from 51 normal human tissues show that sRANKL mRNA is expressed in normal human bone marrow and white blood cells. Methylation status of sRANKL mRNA promoter in 5 lymphoblastoid cell lines (LCLs) signifies that the sRANKL promoter remains heavily methylated in these cell types. TNFSF11 TF ChIP-seq data show that 5 of 161 TFs can bind to the TE-derived sRANKL mRNA promoter region. Four of the five TFs (EBF1, PAX5, IKZF1, and PU.1) bind to this genomic segment exclusively in LCLs, signifying a cell-type-specific transcriptional regulation. Notably, all 4 TFs are known to play a major role in normal and/or malignant lymphopoiesis. Furthermore, IKZF1 and PU.1 represent direct targets of immunomodulatory drugs (IMiDs) for down-regulation.

Summary/Conclusions: Transcription of sRANKL mRNA is driven by a retroviral promoter which remains heavily methylated, thereby inactive, in normal lymphocytes. Epigenetic derepression of this promoter during the course of myeloma development is suggestive of a 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 the sRANKL mRNA promoter in 5 lymphoblastoid cell lines (LCLs) signifies that the sRANKL promoter remains heavily methylated in these cell types. TNFSF11 TF ChIP-seq data show that 5 of 161 TFs can bind to the TE-derived sRANKL mRNA promoter region. Four of the five TFs (EBF1, PAX5, IKZF1, and PU.1) bind to this genomic segment exclusively in LCLs, signifying a cell-type-specific transcriptional regulation. Notably, all 4 TFs are known to play a major role in normal and/or malignant lymphopoiesis. Furthermore, IKZF1 and PU.1 represent direct targets of immunomodulatory drugs (IMiDs) for down-regulation.

E1237

ADENOSINE IN THE MULTIPLE MYELOMA BONE MARROW NICHE: IMMUNE MODULATION 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 ectoenzyme, CD38 catalyzes the initial disassembly of NAD (to cADPR and ADPR), which is followed by adenosinergic activity, provided that CD38 is operating in the presence of other ectoenzymes (CD203a and CD73).

Aims: To demonstrate that adenosinergic pathways contribute to customize homeostasis in MM.

Methods: Evaluation of the expression of adenosinergic 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 other high-risk monoclonal gammopathies. Furthermore, IFNα and PU.1 represent direct targets of immunomodulatory drugs (IMiDs) for down-regulation.
TREATMENT OPTIMIZATION FOR MULTIPLE MYELOMA: SCHEDULE-DEPENDENT SYNERGISTIC CYTOTOXICITY OF POMALIDOMIDE AND CARFILZOMIB ON AN IN VITRO AND EX-VIVO MODEL

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Background: In recent years significant progress has been made in the understanding of Multiple Myeloma (MM) biology. These advances have translated into the development of new drugs and a different approach to treatment, which has ultimately translated into an unprecedented rate of complete remissions. Immunomodulatory drugs (IMiDs) and proteasome inhibitors (Pis) form the backbone of modern MM treatment, but new and more targeted treatments are under development and are being tested in the context of clinical trials. Pomalidomide (POM) is a third-generation IMiD with immunomodulatory, antiangiogenic, and direct anti-MM activities, and greater in vivo potency than its sister Lenalidomide. Carfilzomib (CAR) is a second-generation irreversible PI that is structurally and mechanistically distinct from Bortezomib. Preclinical study suggested that the timing and dosing schedules of IMiDs in combination with Pis treatment is critical, proposing a first evidence that established treatment regimens need to be carefully re-evaluated to maximize the anti-tumor effects.

Aims: In this study we tried to optimize the anti-MM therapy using the new class of agents of IMiDs and new generation PIs, by evaluating a possible synergistic effect between POM and CAR.

Methods: For the purpose of this study we used five bona fide MM cell lines (MM1.S, OPM-2, NCI-H929, KMS12.8M, and U266), a human bone marrow stromal (BMS) cell line (HS-5 cells) and primary samples from newly diagnosed MM patients. Apoptosis analysis was done up to 48h after administration of the first drug. For each drug, three different concentrations were used: low dose, intermediate dose and high dose. Since the BM microenvironment is a complex and active system, with potential contributions of both physical adhesion and soluble factors, we used three experimental conditions to differentiate these interactions: 1) MM cells cultured in complete medium, 2) MM cells suspended in medium conditioned in the prior presence of BMSCs, or 3) MM cells co-cultured with BMSCs in a transwell system.

Results: Using the median effect method of Chou Talalay, we evaluated the combination indices for simultaneous and sequential treatment schedules, and we found that the schedule of administration is important to maximize the synergistic effects. Indeed, schedule-dependent synergistic cytotoxicity was demonstrated for the combination of IMiDs and Pis and a maximal apoptosis consistently observed in IMiDs pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models. Our data overall suggest that the administration of IMiDs before Pis can improve efficacy. Clinical trials are needed to investigate the most effective schedule, which could be to start the administration of IMiDs a day before Pis to increase cells killing.

Summary/Conclusions: Schedule-dependent synergistic cytotoxicity was demonstrated for the combination of CAR and POM and a maximal apoptosis consistently observed in POM pre-exposure schedule. The superiority of this schedule was maintained throughout BM microenvironment models using low dosage of both drugs. Whilst the clinical efficacy of CAR and POM combinations has been demonstrated, the synergistic cytotoxicity may be further exploited by using optimized schedule. Utilizing such a schedule with IMiDs pre-treatment may improve the depth and duration of response of MM patients both as upfront therapy and in the relapsed/refractory setting.

Myeloma and other monoclonal gammopathies - Clinical

E1239

ASSESSMENT OF THE IMPACT OF POST-AUTOLOGOUS STEM CELL TRANSPLANT MAINTENANCE THERAPY ON SURVIVAL OUTCOMES IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA IN THE COMMUNITY-BASED CONNECT MM REGISTRY

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Background: Randomized phase 3 clinical trials have shown that maintenance therapy after autologous stem cell transplant (ASCT) can extend time to progression, progression-free survival (PFS), and overall survival (OS) for patients (pts) with newly diagnosed multiple myeloma (NDMM) (Sonneveld, J Clin Oncol, 2012; McCarthy, N Engl J Med, 2012; Attal, N Engl J Med, 2012; Palumbo, N Engl J Med, 2014; Attal, ASCO, 2016). Connect MM is a largely community-based, US prospective observational cohort study designed to characterize diagnosis, treatment patterns and outcomes in pts with NDMM in clinical practice.

Aims: The Connect MM registry was used to assess impact of maintenance therapy on survival outcomes in pts with NDMM receiving ASCT.

Methods: Adult pts with NDMM were eligible to enroll in the registry within 60 days of diagnosis. Pts were enrolled in 2 sequential cohorts and were treated at the clinician’s discretion as per standard of care. Cohort 1 pts receiving induction and ASCT were included in the analysis and characterized into 4 maintenance regimen subgroups: no maintenance, lenalidomide (LEN)-based maintenance, bortezomib (BORT)-based maintenance, and LEN+BORT maintenance. Duration was from 100 days post-ASCT (no maintenance group) or start of maintenance until progressive disease, death, discontinuation, or data cutoff of January 7, 2016. End points were PFS, second PFS, OS, and safety. An exploratory analysis of the impact of baseline characteristics on survival outcomes was performed.

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 these, 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 improvements across subgroups (age, ECOG status, International Staging System stage, risk group, and induction regimen). No new safety signals were observed.

Summary/Conclusions: In this observational study, post-ASCT LEN maintenance therapy significantly improved PFS and OS compared to no maintenance. These improvements appeared to be independent of induction regimen. Preliminary analysis of second PFS suggests no adverse impact of maintenance treatment on the efficacy of second-line therapy. These data, from a largely community-based setting, support results from randomized phase 3 trials.
E1240
DARATUMUMAB-BASED COMBINATION THERAPIES IN HEAVILY-PRE-TREATED PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA
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Background: Daratumumab-based combination Therapies (DCT) with bortezomib (V)/ lenalidomide (R)/ pomalidomide (P) and dexamethasone (d) have shown exceptional activity in relapsed and/or refractory multiple myeloma (RRMM) in trials. Experience outside of trials since the approval of Daratumumab (D) in 2015 is limited.

Aims: We aimed to review the outcomes of patients who received DCT at our institution.

Methods: Records of RRMM patients seen at Mayo Clinic, MN from December 2015 – December 2016 were reviewed. Patients who received ≥ 1 cycle of DCT were included. Time-to-event analyses were done from date of starting DCT using Kaplan Meier method. Common terminology criteria for adverse events v4.0 were used to grade toxicities.

Results: Of 130 patients, 59% were males and median age at DCT initiation was 67 (49-93) years. ECOG performance score was ≥2 in 29%. Patients were classified as mSMART high (22%), intermediate (22%) or standard (56%) risk. Median time from diagnosis to initiation of DCT was 51.3 (5-156) months, and median number of prior therapies was 4 (1-14). Eighteen (14%) of patients were refractory to prior daratumumab monotherapy. Fifty-three (41%), 34 (26%) and 25 (19%) received DPd, DRd and Dvd respectively. Eighteen (14%) patients received ‘other’ DCT. Median time to first response (≥ PR) was 17%, with clinical benefit rate of 62%. Median estimated follow up from initiation of DCT was 5.5 months (CI 4.2-9.1). The median duration of response was 6.1 months (CI 5.1- not reached (NR)). Median progression free survival (PFS) was 5.5 months (CI 4.1-7.8) (figure A) and median time to next therapy (TTNT) was 5.9 months (CI 4.6-9.4) (figure B). Median PFS for DPd, DRd, Dvd and other DCTs were 4.6 (CI 2.7-NR), 7.8 (CI 5-NR), 3.9 (CI 2.1-NR) and 3.9 (CI 2.8-8.2) months, respectively (p=0.3). Median overall survival (OS) from starting DCT was NR (CI 11.4-NR) (figure C). Median PFS for quadruple refractory (n=28) MM was 2.8 months (CI 2.2-5.3) vs 5.9 months (CI 4.9-NR) for the rest (p=0.008) (figure D). Grade 3 or higher hematological toxicities were seen in 42% of patients. Other toxicities included infections (37%), fatigue (31%), infusion reactions (16%) and diarrhea (10%).

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 this heterogenous disorder. The International Myeloma Working Group recommend low-dose whole body computerised tomography (LDWBCT), PET-CT or whole body magnetic resonance imaging (WBMRI) as initial imaging modalities.

Aims: To compare WBMRI with PET-CT as initial imaging modalities at diagnosis of myeloma or plasma cell disorders. The International Myeloma Working Group recommend low-dose whole body computerised tomography (LDWBCT), PET-CT or whole body magnetic resonance imaging (WBMRI) as initial imaging modalities.

Methods: Both WBMRI and PET-CT were performed at diagnosis of myeloma or a plasma cell disorder 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, 9 IgA, 2 non-secretory, 4 light chain disease, 2 bicalon myeloma). Nine patients had ISS stage 1 disease, 16 with stage 2 disease and 7 with stage 3 disease (range 0-52). 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, however, at vertebrae L5 (using diffusion weighted MRI imaging) was measured. This showed a strong correlation with the degree of bone marrow infiltration by plasma cells (r=0.64). An ADC of <600mm²/s had a negative predictive value of 93% for a bone marrow plasma cell infiltrate of >60%. There was also a significant difference (p=0.012) in the ADC between those with smouldering myeloma and those with symptomatic disease. It was noted that 9 scans resulted in incidental findings including pneumonia, adrenal lesions and one case of colocolic cancer.

Summary/Conclusions: We have shown no difference in PET-CT and WBMRI in detecting a myeloma defining focal bone lesion, or providing prognostic estimation of bone disease. Using MRI, a measure of the ADC at vertebrae L5 has been shown to be a semi-quantitative parameter that correlates with bone marrow plasma cell infiltration and distinguished between those with smouldering and symptomatic disease. In addition it is noted that whole body imaging has led to incidental findings of further pathology, including an unrelated malignancy, which may lead to useful clinical information or to further investigations and imaging which may not be needed.

E1243 PERSISTENCE OF MINIMAL RESIDUAL DISEASE BY MULTIPARAMETER FLOW CYTOMETRY CAN HINDER RECOVERY OF ORGAN DAMAGE IN PATIENTS WITH ALAMYLOIDOSIS

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Background: In multiple myeloma, Minimal Residual Disease (MRD) demonstrated by multiparameter flow cytometry (MFC) identifies subjects with significant residual disease among those who attain complete response (CR). The role of MRD in 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 immunoelectrophoresis, free light chain ratio <30%). In 9 patients with AL amyloidosis, bone marrow aspirates 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 Canto II (USA) instrument. Data were analyzed using the Inflicty software (Spain). Patients were identified as having residual disease if a discrete population of clonal plasma cells comprising ≥50 events was identified (10⁵ limit of detection).

Results: Twenty-eight patients were tested (7 found to have relapsed at the time of MRD assessment with monoclonal components detectable and MRD+ and 21 satisfied current criteria for CR. Nineteen (90%) had reduced and 9 (44%) had improved cardiac function at diagnosis. More than 2 lines of therapy were required to achieve CR in 7 subjects. Median time to CR was 10 months (range: 3-82). Five patients (62%) had achieved cardiac response and 9 (50%) renal response at the time of CR. The median time from CR to MRD was 30 months (range: 6-148), this was not different in the MRD positive vs negative patients (p=0.14). A median of 1089 (range 256-2500) corresponding to 0.04% (range 0.02-0.3%) plasma cells with abnormal phenotype were detected in patients MRD+. No differences in organ involvement, cardiac and renal stage, type of therapy, number of treatments, and organ response at the time of CR was found between the two groups. Interestingly, improvement of cardiac function correlated better than to the time of CR as observed in all 5 evaluable MRD- patients and in none of the 2 MRD+ patients (P=0.047). Compared to the time of CR, renal response was obtained in 7 MRD- subjects (84%) and in 4 (50%) MRD+ (P=0.153). Overall, further improvement of cardiac or renal function after CR was significantly associated with a lower incidence of MRD+ (P=0.047).

Summary/Conclusions: This proof-of-concept study indicates that 43% of patients with AL satisfying current criteria for CR have detectable MRD. MRD positivity could in part explain persistence of organ damage in patients in CR.

E1244 RATES OF PERIPHERAL NEUROPATHY (PN) IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (RRMM) TREATED WITH CARZOMIZ vs COMPARATORS IN PIVOTAL PHASE 3 TRIALS

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Background: PN is a dose-limiting toxicity for some anti-myeloma agents, such as the proteasome inhibitor bortezomib (V).

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.

Methods: This analysis evaluated PN rates in ASPIRE (K [27mg/m²] lenalidomide [R]-dexamethasone[d] [KRd] vs Rd in relapsed or refractory MM; Stewart 2015) and ENDEAVOR (Kd [K 56mg/m²] vD in RRMM; Dimopoulos 2016). We evaluated treatment emergent grade ≥2 PN, patient reported outcomes (PROs; QLQ C30 pain, FACT/GOG neurotoxicity subscales), and progression free survival (PFS) in patients with a baseline history of PN (patients with grade ≥3 PN at baseline or grade 2 PN with pain at baseline were excluded from the studies).

Results: In ASPIRE, grade ≥2 PN rate was low (8.0% [KRd] vs 8.0% [Rd]; Table). Pain subscale scores were similar between arms. Median PFS was longer with KRd vs Rd for patients with grade 2 PN at baseline. In ENDEAVOR, grade ≥2 PN rate during the study (prespecified key secondary endpoint) was significantly lower with Kd vs Vd (6.0% vs 32.0%; Table). Patients had significantly improved pain and neurotoxicity subscale scores with Kd vs Vd. PFS improved with Kd vs Vd in patients with baseline history of grade ≥2 PN (Table 1).

Table 1.

Summary/Conclusions: In ENDEAVOR, the rate of PN was significantly lower with Kd then with Vd; in ASPIRE, PN rate was similar for KRd and Rd. Improved pain and neurotoxicity subscale scores with K may be attributed to better disease control and/or lower PN rates.

E1245 EARLY RELAPSE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN MYELOMA IS A POOR PROGNOSTIC MARKER FOR OVERALL SURVIVAL AND IS DIFFICULT TO PREDICT AT DIAGNOSIS OR DURING INDUCTION TREATMENT

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Background: High dose chemotherapy followed by autologous stem cell transplant (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 50 x 10^9/L and platelets >20 x 10^9/L. Post-transplant PFS & OS was calculated by time (months) from diagnosis to progression or death.

Results: 443 myeloma patients were identified, median age was 57 (r 31-73), 56% were male. 41% of patients were ISS stage 1, 34% stage 2, 25% stage 3. Cytogenetic data was available for 139 patients. 1st-line therapy prior to transplant included an 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 relapse). No statistical difference was found between <12 or >12m relapse for: age, gender, 1st line therapy, ISS stage, Hb, LDH, Ca or cytogenetics, confirming that this group is difficult to predict at baseline. Median OS from time of diagnosis was 103 months (95% CI 101 -137), median OS from start of ASCT was not reached, however 5-year OS was 68%. Patients with progressive disease within 12 months of ASCT, has significantly reduced median OS (median OS:12-31 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:89 months (95% CI 79-98m) p<0.0005. 1st line therapy did not influence likelihood of PFS<12months, with no statistical differences 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 x 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 stromal 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 WALDENSTROM MACROGLOBULINEMIA (WM)

To prospectively collect PROs from the iNNOVATE substudy to assess patients’ perspectives of the therapeutic benefit of ibr, a first-in-class, once-daily inhibitor of BTK, is indicated in the EU for the treatment of WM after ≥1 prior therapy or >12m relapse for: age, gender, 1st line therapy, ISS stage, Hb, LDH, Ca or cytogenetics, confirming that this group is difficult to predict at baseline. Median OS from time of diagnosis was 103 months (95% CI 101 -137), median OS from start of ASCT was not reached, however 5-year OS was 68%. Patients with progressive disease within 12 months of ASCT, has significantly reduced median OS (median OS:12-31 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:89 months (95% CI 79-98m) p<0.0005. 1st line therapy did not influence likelihood of PFS<12months, with no statistical differences 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 x 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 stromal 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.

E1247

INCIDENCE AND RISK FACTORS OF CARDIOVASCULAR ADVERSE EVENTS IN A LARGE POPULATION OF NEWLY-DIAGNOSED, TRANSPLANT INELIGIBLE MYELOMA PATIENTS TREATED WITH CARFILZOMIB


Background: Cardio-vascular (CV) toxicities in patients (pts) with multiple myeloma (MM) may derive from comorbidities, MM itself and its treatment. Carfilzomib, an irreversible proteasome inhibitor, is approved as single agent or in combination with dexamethasone or lenalidomide-dexamethasone for relapsed MM.

Aims: We conducted an integrated analysis of CV adverse events (AE) in newly diagnosed, transplant-ineligible MM patients treated with Carfilzomib in 3 phase III studies (IST-CAR-506, IST-CAR-561, IST-CAR-601).

Methods: All pts were treated with 9, 28-day induction cycles with carfilzomib, cyclophosphamide (300mg/m² on days 1,8,15) and dexamethasone (40mg weekly) (CCyd), followed by carfilzomib maintenance until progression or intel-

Table 1.

Results: Persistent fatigue was the main indication for treatment in 22/31 (71%) pts. Baseline PRO scores were lower for time substudy 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 (≥ 67 points; 84%), and EQ utility scores (≥ 0.08 points; 88%). Time to clinically meaningful improvement was prompt (1 mo for TS and AS; 2 mo for EQ), corresponding with a 48% decline in median IgM (median 20 g/L) after 4 weeks. In pts with baseline anemia (hemoglobin [Hb] ≤ 110 g/L), sustained Hb improvement increased with depth of response. At week 65, Hb levels significantly correlated with TS (r=0.507, P=0.01) and AS (r=-0.519, P=0.008), and were marginal for EQ (r=0.39, P=0.054). Although IgM levels did not significantly correlate with PRO scores, the benefit was similar in responders regardless of depth of response.

Table 1.
erance. Carfilzomib was administered i.v. at the dose of 36mg/m² on days 1, 2, 8, 9, 15, 16 in the IST-CAR-506 trial: at 3 dose levels escalated from 45 to 70mg/m² on days 1, 8, 15 in the IST-CAR-561 trial and on days 1, 2, 8, 9, 15, 16 in the IST-CAR-601 trial. AE's were graded based on NCI-CTCAE v4.

Results: 148 pts with a median age of 72 years were analyzed. At enrollment, 34% of patients had at least 1 cardiovascular risk factor; 20% had peripheral vascular disease (including hypertension in 13% patients), 19% diabetes and 5% chronic pulmonary disease. After a median follow-up of 21 months, at least 1 any grade CV-AE occurred in 45% of patients; any grade hypertension was reported in 17% of patients, dyspnea in 9%, and heart failure, arrhythmia and venous thromboembolism (VTE) in 6% of patients, each. Grade 3-5 CV-AEs occurred in 15% of patients, the most common being heart failure (4%), hypertension (3%), pulmonary edema (3%) and VTE (3%). Four (3%) fatal CV-AEs occurred: 1 case of heart failure, pulmonary edema, arrhythmia and VTE, respectively. No difference in terms of CV-AEs was observed in patients treated with different doses of carfilzomib. In pts who developed at least 1 CV-AE, carfilzomib dose reduction (33%) and discontinuation (33%) were more frequent as compared to those without CV-AEs (12% and 18%, respectively; p<0.0001).

A trend toward a shorter 2-year overall survival (adjusted for age) was observed among patients who experienced at least 1 CV-AE as compared with those who did not (74% vs 83%; HR: 0.51; p=0.066). Pts ≥75 years had a higher risk of any grade (58% vs 36%; p=0.02) and grade 3-5 CV-AEs (34% vs 15%; p=0.01). major cardiac events of any grade were more frequent in older patients (29%) than in younger ones (6%; p<0.001). Patients with at least 1 CV risk factor at enrollment had a 4-fold increased risk (odds ratio: 3.79; p<0.001) of developing a CV-AE during treatment as compared to patients with no CV risk factors; in detail, baseline hypertension (odds ratio: 4.12; p=0.012) and peripheral vascular disease (odds ratio: 3.75; p=0.002) conferred the highest risk of developing CV-AEs.

Figure 1.

Summary/Conclusions: Among newly diagnosed MM pts treated with carfilzomib, cyclophosphamide and dexamethasone, at least 1 CV-AE occurred in 45% of pts, hypertension and dyspnea were the most predominant. Pts ≥75 years of age and those with at least 1 pre-existing CV risk factor were at higher risk of developing a CV-AEs. The onset of CV toxicity significantly increased the rate of dose reductions and treatment discontinuation, translating into higher risk of death. CV toxicity may significantly impact on treatment compliance and survival. Therefore, to derive maximum benefit from Carfilzomib, all pts - particularly the elderly - should be carefully assessed to select the most appropriate treatment.

E1249

POMALIDOMIDE (POM) + LOW-DOSE DEXAMETHASONE (LODEX) AFTER SECOND-LINE LENALIDOMIDE (LEN)-BASED TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA (RRMM): UPDATED PROGRESSION-FREE SURVIVAL ANALYSIS

D.S. Siegel1, G.J. Schiller2, K. Song3, R. Agajanian4, K. Stockerl-Goldstein5, 69.4% for pts ≥ MR, and 69.1% for pts with ≥ PR. In addition, pts with ≥ MR had similar treatment durations as those achieving ≥ PR (10.5 vs 11.5 months; Table). Common grade 3/4 adverse events (AEs) included anemia (25.5%), neutropenia (11.8%), and infections (19.6%; including pneumonia [9.8%]. No pts experienced SPMs. In the immune subset analysis, the proportions of CD3+ and CD3+CD8+ T cells after treatment were significantly higher vs baseline (72.6% vs 67.8% and 36.9% vs 32.1%, respectively; P<0.05). Pts with response also had significantly elevated proportions of these T-cell populations, but pts with no response did not. Relative changes from baseline for CD3+ and CD3+CD8+ T-cell populations were significantly greater in pts with response vs those with no response (10.4 vs -0.8 and 4.2 vs –3.5, respectively; P<0.05).

Table 1

Summary/Conclusions: This update confirms the safety and efficacy of POM + LODex following second-line LEN-based treatment failure in pts with RRMM. Hematologic AE rates improved, and median PFS was longer with third-line use than previously reported with POM + LODex use in later treatment lines. In addition, achieving disease control of ≥ MR led to similar PFS rates as reaching ≥ PR.

E1249

“REAL WORLD” DATA ON THE EFFICACY AND SAFETY OF IKAZOMIB IN COMBINATION WITH CARFILZOMIB 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). This was a retrospective, non-interventional study, which recorded IrD treatment data from patients with RRMM who participated in the name-patient program of ixazomib in Greece. The primary endpoint was the evaluation of overall response rate (ORR) following treatment with IrD. 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.

Methods: Forty-one patients were included in the present study. Of those, 35 (19M/16F, 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),
5.7% (2/35) and 2.9% (1/35) had received 2 and 3 prior treatment lines, respectively. Overall, 82.9% (29/35) of patients had been exposed to proteasome inhibitors prior to IRd (77.1% to bortezomib and 8.6% to carfilzomib) and 46.8% (17/35) to IMiDs [31.4% (11/35) to thalidomide and 22.9% (8/35) to lenalidomide]. Autologous transplantation had been given in 42.9% (15/35) of patients. Median treatment duration was 7.1 months. Among 34 patients with available data, median VEGF was decreased in 15.7% (5/31) of patients, 43.8% (14/31) of patients, and 52.9% (16/31) of patients in the LEN, BORT, and THAL cohorts, respectively. The incidence of SPMs per 100 patient-years was 3.87 overall and 3.45, 5.41, 2.73, and 6.51 for LEN, BORT, THAL, and other agents, respectively. Median time to best response was 1.2 months. Treatment interruptions due to AEs were recorded for 11.4% (4/35) of patients, while 20.0% (7/35) of patients discontinued treatment. Reasons for ixazomib discontinuation were AEs for 3 patients (an event of myelodysplastic/myeloproliferative disease), discontinuation in discussion with the patient and, administrative reason (patient could no longer visit the site) in one. Among the 35 patients analyzed, 17.1% (6/35) had experienced disease progression or death; the 6-month PFS rate was 90.5% and the 6-month TTP rate was 93.2%. Regarding AEs of interest, 31.4% (11/35) of patients experienced peripheral neuropathy (10.9%, 4.6%, and 4.6% for LEN, BORT, and THAL, respectively), which did not resolve; while 45.4% (5/11) had not resolved (three were grade 1, one grade 2, and one of grade 3) at the end of follow-up. In addition, 31.4% (11/35) of patients developed gastrointestinal AEs, 11.4% (4/35) experienced pneumonia, 9.8% (3/32) hypotension, 5.7% (2/35) cataract and herpes zoster, and 2.9% (1/35) deep vein thrombosis; no cardiac arrhythmia or other cardiac events were recorded, while osteonecrosis of the jaw developed in 5.7% (2/35) of the patients.

Summary/Conclusions: This study showed that the IRd regimen produces an ORR of near 68% and a clinical benefit in almost all patients with RRMM who are treated in RW practice. IRd acts rapidly and has an acceptable toxicity profile with no cardiac events.
Results: During the development process, a number of similarities and discrepancies between centers as well as evidence gaps were identified. Intense discussion and literature searches resulted in a concise, harmonized clinical pathway, released by all 14 Centers of Excellence. This is freely available on the website cc-netzwerk.de and provides a very decisive insight according to the current state of knowledge on the CCC-level (e.g. on the diagnostic algorithm, Fig. 1). The clinical pathway is well suited for informing patients and physicians about the most up-to-date, comprehensive medical treatment standards as well as innovative procedures. Furthermore, this project initiated the idea of developing a national evidence-based clinical practice guideline for MM in the frame of the German Guideline Program on Oncology.

Figure 1.

Summary/Conclusions: The first clinical MM pathway developed and released by all 14 German CCCs/Centers of Excellence translates scientific evidence and expert knowledge into precise suggestions for MM patients in clinical practice. These measures might increase the quality of cancer care throughout Germany. To be up-to-date and to reflect latest research findings this clinical pathway will be updated every 18 months.

E1252

WT1 HETEROCLITIC EPITOPE IMMUNIZATION FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH HIGH-RISK MULTIPLE MYELOMA (MM)

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Background: The Wilms tumor 1 (WT1) protein is a tumor associated antigen that is a target for anticancer immunotherapy. We had previously demonstrated overexpression of WT1 in multiple myeloma (MM) cells by IHC, as well as for formation of a WT1 peptide fragment (RMFPNAPYL)/HLA-A*0201 complex on the engagement interface between malignant plasma cells and T-cells in HLA-A*0201+MM pts using the high-affinity fully human IgG1 mAb ESK1. We report initial results from MM pts immunized with the WT1 heteroclitic peptide mixture galinpepimut-S (GPS) after autoSCT.

Aims: To determine the safety and potential efficacy of the WT1 heteroclitic peptide mixture galinpepimut-S (GPS) after autoSCT.

Methods: 16 MM pts underwent autoSCT with melphalan conditioning followed by (fb) lenalidomide maintenance starting 3 months (mos) post-SCT. 13/16 pts presented with high-risk (HR) cytogenetics [t(4;14), t(14;16), del17p, 1q21/22 gain and/or del13q]. GPS was administered in patients with mounting s.c. starting 2 ws post-SCT and q2 ws thereafter x 6 initial doses fb boosters q4 wks x 6 additional doses. GM-CSF was given on days -2 and 0 of each cycle.

Results: Various levels and were induced not only against the heteroclitic peptides (within GPS), but also against the corresponding native WT1 peptide sequences as well as the ‘total pool’ of WT1-derived overlapping peptides.

Summary/Conclusions: Administration of the novel WT1 heteroclitic peptide immunizer GPS post-SCT demonstrates favorable safety profile along with an encouraging mPFS of currently 23.6 mos in this high-risk MM population. This ‘off-the-shelf’ immunotherapy is easy to administer and has been specifically designed to elicit responses across most common HLA Class I and II alleles. Based on these results, a larger phase II trial is being planned to optimally integrate post-transplant immunotherapeutic strategies to meaningfully delay or reduce risk of relapse in this challenging clinical setting.

E1253

ANALYSIS OF MULTIPLE MYELOMA PATIENTS WITH PROGRESSIVE DISEASE AT TIME OF FIRST AUTOLOGOUS STEM CELL TRANSPLANTATION: PREDICTORS OF PROGRESSIVE DISEASE AND FACTORS AFFECTING SURVIVAL

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Background: The impact of response depth at time of autologous stem cell transplantation (ASCT) on the progression-free and overall survival (PFS and OS, respectively) of multiple myeloma (MM) patients has been an extensively investigated area. Rosiñol et al. (2011) reported a significantly worse PFS and OS in patients with progressive disease (PD) compared to stable disease (SD) at time of ASCT; Parrish et al. (2015) stated a significantly shorter PFS, but not OS, in patients with PD in comparison to SD or minimal response (MR) at time of ASCT.

Aims: Our goal was to characterize MM patients with PD at time of first ASCT. We aimed to analyze the effects of PD on PFS and OS. Next, we intended to examine the influence of PD at time of ASCT (evaluated at beginning of induction therapy as well as use of novel agents in induction therapy, response after 1st ASCT, and use of maintenance therapy in those patients to identify predictors for PFS and OS. Furthermore, we aimed to identify clinical/therapeutic features predicting the occurrence of PD before ASCT.

Methods: Non-trial MM patients who had undergone a single or tandem ASCT at the University Hospital of Heidelberg in the years 1992-2014 were analyzed regarding their impact on PD before 1st ASCT. We performed an analysis of PD at time of ASCT and before ASCT. PFS was calculated from date of 1st ASCT, except for prognostic impact of response assessment after 1st ASCT, where date of response assessment was used. Start of maintenance therapy was analyzed as time-dependent factor.

Results: Non-trial patients transplanted in our center between 1992 and 2014 were compared. Patients with PD ≤100 days before ASCT had similar PFS and OS as non-PD patients. Neither the clinical parameters at induction start, response after 1st ASCT, nor maintenance therapy had a significant effect on PFS in those patients. In the univariate analysis, high-risk cytogenetics as well as elevated LDH at induction start had a significantly negative effect on OS in patients with PD before ASCT. Response was evaluated according to 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.

Conclusion: Our analysis of 51 patients with PD at time of ASCT among 874 ASCT-patients with MM transplanted between 1992 and 2014 shows no significant difference regarding PFS and OS between patients with PD and other response rates. It was further shown that high-risk cytogenetics as well as elevated LDH at beginning of induction therapy have a significant impact on worse OS in patients with PD at time of ASCT. In addition, high-risk cytogenetics at time of ASCT is a significant factor for occurrence of PD. The impact response depth at time of ASCT is not entirely clear, especially regarding the benefit of ASCT in patients with PD at time of ASCT, as reports from other centers show significantly worse PFS and OS (Rosiñol et al.), or only PFS (Parrish et al.) in patients with PD at time of ASCT.
Multiple myeloma (MM) is a disease of the elderly. The prevalence 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, deep vein thrombosis, and pulmonary embolism, 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 years with Charlson Comorbidity Index 2±0.5), median duration of treatment 192±54 days. 42% (n=1779) were treated with PIs, 38% (n=1624) with IMiDs and 20% (n=865) with PI+IMiDs. Patients receiving PI+IMiD were significantly younger and generally had lower prevalence of CV comorbidities compared to those receiving PI or IMiD (Table). Compared with patients on PI, the risk of developing VTE was 46% greater in patients on PI+IMiD (HR 1.46; 95% CI: 1.09, 1.96). Compared with those on IMiD, the risk of developing cardiac failure and cardiac arrhythmia was 33% and 18% greater in patients on PI+IMiD (HR 1.33; 95% CI: 1.03, 1.72; HR 1.18; 95% CI: 1.00, 1.40). After 6 months of treatment, the rates of VTE were 8%, 10%, and 11% for patients on a PI, those on an IMiD and those on PI+IMiD, respectively. The corresponding rates for cardiac failure were 18%, 11% and 11% for PI, IMiD and PI+IMiD cohorts, and 21%, 16% and 22% for cardiac arrhythmia.

Summary/Conclusions: PI+IMiDs may be associated with incremental occurrence of specific CV events during treatment, and may result in specific CV events earlier during therapy than PIs or IMiDs alone. These highlight a need for treatments that do not exacerbate CV risks and are appropriate for patients with pre-existing CV conditions. The lower prevalence of baseline CV comorbidities and lower mean age in patients on PI+IMiDs suggest that prevalence of a CV comorbidity and age influences treatment choice. Further analysis may be necessary to better understand the impact of baseline CV comorbidities on choice of MM treatment.
**Methods:** We collected the clinical data of 169 patients qualified to therapy/oncology centers. VTD protocol recommended by Polish Multiple Myeloma Group and local preferences. Observations from routine practice might have though not to align with those of ELOQUENT-2.

**Aims:** We performed a historical comparison based on a systematic review of 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 6192 registry results, 7 studies (8 publications) met the inclusion criteria (4 Ld studies, 3 LD studies). Data for overall survival and tolerability from 1153 patients in the Ld group and 353 patients in the LD group were analyzed. The median patient age was 63–68 years. Most patients were white, male and had an ECOG score of 0.

**Summary/Conclusions:** Overall survival and safety are not significantly affected by different dosing of dexamethasone in combination with lenalidomide; thus, use of Ld or LD should be based on the patient population. Further studies may provide additional evidence to inform clinicians and revision of international guidelines for dexamethasone dosing in RRMM.
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 isoform by immunofixation at diagnosis was as follows: light chains only (46.4%), IgG (39.1%), IgA (10.2%) and IgM (4.3%). The predominant light chain isoform was lambda (79.7%). A very good partial response (VGPR) or better was achieved in 53.6% of patients. Three-year OS rate was 64.3%. 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. 9; P<0.001). Also a trend towards a higher difference between involved and uninvolved free light chains was observed in the group of patients with IP (360.2 vs. 221.7; P=0.08). IP was more frequent in those who received an ASCT (57.9% vs. 42.1%; P=0.03). There were no changes in the frequency of IP comparing at diagnosis vs. post-ASCT settings (39.13% vs. 34.78%; P=0.4).

Regarding its prognostic value, IP did not influence survival in the whole series. In the ASCT group, the presence of IP resulted in a significantly shorter PFS (median: 30.2 months vs. NR; P=0.019; Figure 1A) and OS (62.5 months vs. NR; P=0.097). In the group of patients with stage I and II of the Mayo risk stratification system of 2012 (Mayo12), the presence of IP resulted in a shorter PFS (21.8 months vs. NR; P=0.047; Figure 1B), but not significantly different in OS. Multivariate analysis restricted to the population of patients with stage I and II of Mayo12, incorporating ASCT, BMPC and IP, indicate that IP retained its independent prognostic factor for worse PFS (HR=12.05; 95% CI, 1.9-75.7; P=0.008).

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.

Table 1.

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 (MiMDs) 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≥60 days or retreatment following a treatment gap>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 356 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 bortezomb (B)-based regimens (29.4%) and other regimens not containing novel agents (35.2%). Other regimens included 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 15.6%, 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 statistical significant 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: Several studies have shown that serum free light chain (sFLC) concentrations, in particular the ratio of sFLC lambda/kappa (L/D) or sFLC light chain ratio (L/L+D), have been associated with outcome in multiple myeloma (MM) patients.
Background: Polyclonal antibodies against the conformational epitopes between the heavy and light chains (HLC) of immunoglobulin (Ig) 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 Ig, allowing the quantification of the monoclonal component. HLC and k/λ 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 with oligo-secretory disease. Our goal was to evaluate the diagnostic and prognostic role of HLC ratio in patients achieving complete response (CR) after treatment.

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 lambda isotype was IgG λ or IgA λ 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 (AuStC) received a bortezomib-based treatment mainly constituted by bortezomib, melphalan and prednisone combination (VMP), while eleven patients underwent AuStC 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 14-45). CR was significantly associated with better PFS (43 months, 95% CI 14-45) vs 35 months (95% CI 10-38), p=0,049. In contrast, normalization of HLC ratio had no impact on PFS (26 months, 95% CI 10-38) and progression-free survival (PFS) was 26 months (95% CI 14-45) vs 35 months (95% CI 10-38), p=0,049. Neither FLC, nor HLC affected OS. There were no differences between patients who received AuStC and those who did not.

Summary/Conclusions: To the best of our knowledge, this is the first study to analyze HLC ratios exclusively in MM patients in CR. While our preliminary data confirm the prognostic usefulness of FLC in this setting, currently they do not support a role for HLC as putative biomarker of MRD.

E1262
REAL-WORLD RESULTS OF DARATUMUMAB MONOTHERAPY IN HEAVILY PRETREATED RELAPSED/REFRACTORY MULTIPLE MYELOMA IN POLAND: A PROSPECTIVE OBSERVATIONAL STUDY OF THE POLISH MYELOMA GROUP

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Summary/Conclusions: In this first real-world analysis we confirm that daratumumab monotherapy is able to induce response in one third of highly pretreated and double refractory RRMM patients. Regarding safety, in contrast to the SIRIUS trial where no treatment discontinuations due to 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

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Background: Emerging resistance to modern antimyeloma agents such as proteasome inhibitors (PIs) and immunomodulatory drugs (IMDs) remains the main clinical problem in managing multiple myeloma (MM). Daratumumab, a first-in-class anti-CD38 monoclonal antibody, has been recently approved in Europe as a monotherapy for patients (pts) with relapsed and refractory multiple myeloma (RRMM), whose prior therapy included a PI and an IMID and whose disease had progressed during this time; based on the SIRIUS clinical trial outcome (Lonial et al. Lancet 2016). Nevertheless to optimize daratumumab use in clinical practice, more data on its “real life” activity and safety are still required.

Aims: This observational study of the Polish Myeloma Group (PMG) was aimed to prospectively evaluate the efficacy and toxicity of daratumumab monotherapy in RRMM patients treated in Poland (DaraCUP).

Methods: Patients were eligible for DaraCUP if they met all the following criteria a) confirmed diagnosis of RRMM, b) relapse after a minimum of 3 prior lines of therapy, c) were double refractory (PI and IMID) or were double refractory (PI and IMID) and had a ECOG performance status score 2 or lower. Data on treatment outcomes and complications were anonymously collected using electronic CRFs. The IMWG response criteria were applied.

Results: In total 30 patients were qualified to DaraCUP in Poland and all were enrolled to the PMG observational study. At the time of writing this report, 26 pts (87%) had received at least one dose of daratumumab and were included in the safety analysis, while 22 pts (73%) had received at least 2 cycles of daratumumab and were included in the preliminary efficacy analysis. Baseline pts characteristics are reported in Table 1. Pts were heavily pretreated, with a median of 4 prior lines of therapy (range, 2-10). Ten pts (38.5%) were double refractory to both PI and IMID while 15 pts (53%) were refractory to the last line of previous therapy.

Median time since initial diagnosis to start of treatment with daratumumab was 3.9 years (range, 1.4-12.2 years). At the time of analysis, the median follow-up time within the study was 5.1 months (range, 0-8 months) and median cumulative treatment duration was 4.5 months (range, 7-8 months). Sixteen pts (61.5%) remained on treatment, while ten pts (36.5%) discontinued therapy as a result of disease progression (n=7) and adverse events (AEs) (n=3). Overall response rate (PR or better) was 31.8% including one (4.5%) CR and two (9%) VGPR (Table 1). Stable disease was reported in 11 (30%) pts. The median PFS and OS had not been reached. During the treatment, the observed treatment side effects were recorded due to disease progression. Regarding daratumumab toxicity, grade 3 or 4 non-haematological toxicities occurred in 8 pts (30.7%) and included: infection-related reactions (n=2), pneumonia (n=2), other infections (n=2), mandible inamllation (n=1), dyspnoea (n=1). The most common haematological toxicities were grade 4 anemia (n=30.7%) and neutropenia (n=23.1%); while thrombocytopenia occurred in 3 pts; 11.5%. Grade 3 or 4 anaemia and neutropenia were found in 3 (11.5%) and 2 (7.7%) pts, respectively. Updated results will be presented at the meeting.

Table 1.
Background: Multiple myeloma (MM) is the second most common haematological malignancy after non-Hodgkin lymphomas, accounting for 13% of blood malignancies and 1% of all cancers. The medical management of multiple myeloma has changed over the years and is influenced by multiple factors (e.g., evidence from clinical trials, drug approval status, level of drug reimbursement, guidelines), which vary across Europe. Information describing how patients are managed may vary across the real world and may need to be improved.

Methods: Physicians in Europe were requested to answer a series of questions on patient characteristics and treatment regimens of the last eight patients that they had treated during the month prior to answering the questionnaire, according to their patients’ medical charts. The questionnaire was conducted between January and June 2016. Data on 2564 patients with MM were available and are presented here. Countries were grouped into regions according to similar health care systems: Spain, Portugal, Italy and Israel (Southern Region, SR, n=522); Greece, Austria, Bulgaria, Czech Republic, Latvia, Lithuania, Poland, Serbia, Slovakia (Eastern Region, ER, n=689); Analy- ses were descriptive.

Results: Patient characteristics were generally similar across regions, with the majority being >75 years old (69-76%), receiving frontline therapy at study inclusion (57-58%), and being ineligible for autologous stem cell transplan- tation (ASCT) (53-59%). The median time from MM diagnosis to the time that the physician answered the questionnaire was higher in ER (19.5 months) than other regions (9.7-11 months) (Table). The majority of frontline regimens contained bortezomib, although this was lower in ER (51%) than in other regions (66-70%). The median duration of frontline therapy was longer in ER (4.5 months) than other regions (3.2 months). This difference was mainly driven by ASCT eligible patients having longer duration of therapy in ER (4.5 months) than other regions (2.9 months). The number of bortezomib injections in frontline therapy, however, was higher in SR and CRN (both 24) than in ER (18). The majority of second line regimens contained lenalidomide (57-64%) in all regions except ER, where bortezomib-based regimens were most frequent (38%). The median duration of second line therapy was shorter in SR and CRN than in ER (Table). Moreover, second line therapy, ASCT eligible patients had shorter duration of therapy in ER and SR (3.2 months) than in CRN regions (4.5 months). The majority of later-line (3+) regimens were based on therapies that did not include bortezomib, lenalidomide or pomalidomide for all regions (57-67%) with the exception of SR where pomalidomide (29.4%), lenalidomide (12.6%) and bortezomib (14%) were the preferred options. In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.

Summary/Conclusions: In this analysis conducted in Europe, treatment patterns differed in terms of regimens used and duration of therapies even though patient characteristics were generally similar across regions. This may in part be related to differences in healthcare systems. Further research is required to understand how new therapies can be integrated into current treatment patterns and how these treatment patterns impact patient outcomes.

Table 1.

Table 2: Univariate and Multivariate Cox Regression Analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (95% CI)</td>
<td>P-value</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Age years</td>
<td>1.09 (1.05-1.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Charlson Comorbidity index</td>
<td>1.2 (1.05-1.3)</td>
<td>0.005</td>
</tr>
<tr>
<td>Number of Frailty criteria</td>
<td>1.2 (1.14-1.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ISS</td>
<td>2.6 (1.9-3.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Presence of Renal Failure</td>
<td>1.6 (0.99-2.6)</td>
<td>0.054</td>
</tr>
<tr>
<td>Típ de QT</td>
<td>1.38 (0.75-2.53)</td>
<td>0.29</td>
</tr>
<tr>
<td>Alaplanites</td>
<td>1.63 (0.89-2.93)</td>
<td>0.11</td>
</tr>
<tr>
<td>IMiDs</td>
<td>1.23 (0.45-3.37)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This study shows that the prevalence of frailty syndrome is high and has a profound impact in early death. It is also independently associated with a worse prognosis. Frailty should be considered as part of the clinical assessment when treating elderly patients with myeloma.

PROGNOSIS OF AL AMYLOIDOSIS WITH KIDNEY INJURY

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Background: AL amyloidosis is a rare disease related to excessive and uncon- trolled secretion of monoclonal light chains. The consequence of this prolifer- ation is an alteration of the affected organs due to deposition of free light chains. Despite therapeutic advances in recent years based, among others, on the finding of French studies, the prognosis of this disease remains poor in particular for patients with cardiac disease. Kidney involvement is also frequently observed in the form of a classic renal amyloidosis, but at present the prognosis of chronic renal failure in this context is unknown.

Aims: The study was interested in the prognosis of AL amyloidosis associated with endstage renal disease on dialysis in the era of treatment with bortezomib.

Methods: A total of 133 patients (61 from ile-de-France region register and 72 from reference center) were evaluated. Median survival was 63.6 months compared to 70.6 months for patients without dialysis (p=0.65). Within the group
of patients on dialysis, there is no significant difference between those receiving or not bortezomib. Median survival before 2008 was 54.82 months and rose to 82.30 months for patients treated after this date (p=0.95). Age (HR: 0.2819, CI 0.1375 to 0.5782), heart disease (HR: 0.3746, CI 0.1724 to 0.8141) and serum albumin (HR: 2.50 CI: 1.077 to 5.803) were identified as prognostic factors. Transplantation is a viable treatment option for good responders.

**Summary/Conclusions:** Prognosis of AL amyloidosis in dialysis is heterogeneous. Prognostic scoring integrating clinical biological data could identify the patient who may benefit the most dialysis. This results need to be matched by sex and age with non-dialysis and dialysis for another cause.

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**E1267**

**FDG-PET IN MULTIPLE MYELOMA: DUAL TIME POINT FDG UPTAKE IN FOCAL LESIONS CORRELATE TO RESPONSE TO CHEMOTHERAPY**


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**Background:** Dual Time Point (DTP) 18F-FDG PET imaging has been shown to be useful in differentiating malignant from benign lesions in that increasing uptake from 1 to 3 hours is a characteristic feature of malignancy in contrast to inflammation.

**Aims:** The aim of this study was to evaluate the predictive role of DTP 18F-FDG PET/CT imaging in assessing response to chemotherapy in multiple myeloma (MM).

**Methods:** 23 patients with MM (21 male, aged 53-75 years) underwent 18F-FDG PET/CT in a prospective study (NCT02187731) before start of treatment and two months after high dose chemotherapy with stem cell support. All scans were performed at 60 and 180 minutes after tracer injection at Odense University Hospital and Vejle Hospital. Thirteen patients with ≥3 focal lesions of at least 10 mm were selected for analysis. Images were analyzed using an adaptive thresholding algorithm (ROVER software; ABX GmbH, Radeberg, Germany). Focal malignant lesions were localized in pre-treatment scans; maximum standard uptake value (SUVmax) and mean SUV (SUVmean) and partial volume corrected SUVmean (pSUVmean) were obtained for each lesion. 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 lesions with complete response (27.7% vs 11.4%; P=0.050). Additionally, the increase in pSUVmean 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 correlate with patients who achieved a greater response to stem cell support.

The increase in pSUVmean is a better index than that of SUVmean and SUVmax for this purpose.

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**E1268**

**UNDERSTANDING THE CONTRIBUTE OF THE NOTCH PATHWAY IN MULTIPLE MYELOMA BONE MARROW NICHE: A FOCUS ON EXTRACELLULAR VESICLES-MEDIATED COMMUNICATION**

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**Background:** Multiple myeloma (MM) is an incurable cancer stemming from malignant plasma cells. MM is characterized by a strong tropism to the bone marrow (BM), where tumor cells accumulate and establish complex interactions with the normal stroma, which in turn promotes tumour survival, drug resistance and the development of bone disease. The Notch oncogenic pathway provides a key contribute to the ability of MM cells to shape the BM niche, affecting both MM cell biology and the interplay between MM cells and BM. Only extracellular vesicles (EVs) have been reported as novel mediators in creating a supportive milieu for MM. Here we investigate the role of the activated Notch signaling in EV-mediated cross-talk.

Aims: 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.

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**E1269**

**REAL-WORLD DATA ON THE TREATMENT OF RELAPSED/REFRACTORY MYELOMA WITH LENALIDOMIDE AND DEXAMETHASONE IN 2ND LINE (LEGEND STUDY): THE PROGNOSTIC SIGNIFICANCE OF BIOCHEMICAL VS. CLINICAL RELAPSE**


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**Background:** The combination of lenalidomide/dexamethasone (LenDex) is an established treatment for relapsed/refractory Multiple Myeloma (MM) patients; however, apart from clinical trials, there is limited data for the efficacy of this combination as 2nd line treatment. Furthermore, the efficacy of LenDex when administered before evident clinical manifestations, namely in the case of biochemical relapse as compared to clinical relapse, has not yet been assessed.

**Aims:** In the current study, we evaluated response rates and progression-free survival (PFS) in patients treated with LenDex in 2nd line and we compared survival of patients treated with LenDex at biochemical relapse vs those treated at clinical relapse.

**Methods:** Medical files of 207 patients with MM diagnosed between 2000-2013 in 18 Greek centers and treated with LenDex as 2nd line treatment from January 1st 2009, up to March 1st 2014, were retrospectively studied. Overall response and PFS were evaluated for all patients. Additionally, PFS was compared in patients treated at either biochemical relapse (group A) or at clinical relapse (group B). The prognostic significance of biochemical relapse adjusted with important patients' characteristics was also evaluated. Classical methods were used for statistical analysis.

**Results:** Two hundred and seven patient files were recorded and analyzed (M/F: 112/95, median age: 67.2y, range 31-91y; IgG: 115, IgA: 55, Light chain: 11.4%; P=0.050). Additionally, the increase in pSUVmean 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:** Our data confirm that LenDex combination as 2nd line treatment leads to high overall response rates and prolonged PFS. Additionally, we have shown for the first time in routine clinical practice that MM patients who receive 2nd line therapy with LenDex at biochemical relapse have a significantly longer median PFS compared to patients treated at clinical relapse, underlining the importance of potentially starting treatment before evident clinical manifestations at the first relapse.
Methods: We established two MM cell lines stably retaining the doxycycline-inducible pTRIZP vector containing anti-Jagged1 and Jagged2 shRNAs and a BM mesenchymal stromal cell (BMSC) line expressing shRNAs for Notch1 and Notch2. EVs were isolated by ultracentrifugation and used for functional assays and molecular analysis. qPCR was performed using SYBR Green. Apoptosis analysis was performed by flow cytometry; evaluation of protein expression was by Western blot analysis or western transfer. Results: We present evidences that EVs play a crucial role in the dysregulated interactions of MM cells with the BM microenvironment and that Notch regulates their release. Indeed, BMSCs 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 production of pro-tumor factors by BMSCs (i.e. SDF1α), promoting their ability to boost tumor growth; interestingly, this effect is lost when EVs are isolated from MM cells where the Notch pathway was inhibited. Finally, EVs released by co-cultures of BMSCs and MM cells where the Notch pathway is blocked display a reduced ability to increase osteoclastogenesis compared to those 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 de-arranged BM niche represent the rationale for a Notch-directed therapy aiming to uncouple the crosstalk of MM with the surrounding microenvironment by inhibiting Notch signaling.

E1269
THE USE OF CARFILZOMIB AND BORTZEOMIB IN ROUTINE CLINICAL PRACTICE: RESULTS FROM PREAMBLE, AN ONGOING, OBSERVATIONAL COHORT STUDY IN MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) remains largely incurable despite improvements in clinical outcomes following the approval of immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) (Rajkumar et al 2010). Previous findings suggested that time to next therapy (TTNT) was an important predictor of treatment success (Kumar et al 2011). However, the clinical implications of TTNT are not well understood. The aims of our study were to evaluate the clinical implications of TTNT and to describe how patients managed to achieve this. Aims: In this subsequent PREAMBLE analysis, treatment patterns in patients (pts) with RRM receiving bortezomib (bort) and carfilzomib (carf) were evaluated to better understand the use of pts in routine clinical practice.

Methods: PREAMBLE (NCT01838512) is an ongoing, observational, interna- tional 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 treata- ment and initiated treatment (index therapy) with an IMiD, PI or IMiD+PI within 90 days before to 30 days after study enrollment. Treatment patterns, DoT and time to next treatment (TTNT; for pts who switched or died) were assessed. Informed consent was obtained for all pts.

Results: At data cut-off (Sept 1, 2016), data were available for 924 pts, of which 326 (35%) pts had bort-based index therapy and 86 pts (9%) received carf-based index therapy (63/72 [88%] were enrolled in North America). The most common bort-based combination was bort + dexamethasone (dex; n=99; 30%). The most common carf-based therapies were carf alone (n=40; 47%), followed by carf+dex (n=21, 24%). The most widely used bort dose per 21 days for any bort-based therapy was 1.4mg/m2 (28/55; 51%). Switch from carf-based index therapy occurred after a median (Q1, Q3) DoT of 3.4 (1.9, 9.5) mo (n=34); most pts switched to pomalidomide (pom)-based regimens (23/34; 68%). Switch rates increased from 17% at 3 mo to 57% at 33 mos. The most widely used carf dose per 21 days received for any carf-based therapy was ≤120mg/m2 (89/151; 56%). The most common carf dose per 28 days received for any carf-based therapy was ≥120mg/m2 (28/55; 51%). 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 electro- phoresis was performed using Capillaries II, serum and urine immunofixation using Hydrasys II (both from Sebia), sFLC were measured on Immage 800 nephro- elution (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 measure- ment at baseline (at MM diagnosis or first relapse). Serum and urine 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%). sFLC or BJP were not available in 10% of 872 pair of samples from 43 pts. In 10% of cases (68/696 pair of samples) sFLC ratio was abnormal with increased involved FLC with no detectable BJP (FLC+/sFLC+BJP-); the opposite (FLC-/sFLC+BJP+) occurred in 1% of cases (8/696 pair of samples). Renal failure was found in 9/3 13% of discrepant cases. At baseline, of the 43 LCMM pts, 26 had ‘measurable disease’ only by sFLC due to BJP-200mg/24h and were therefore considered not evaluable for response assessment. Median time to BOR was 3 months by both sFLC and BJP and (range: 1C1-11 mesi; range: BJIP-10 mesi). Among the remaining 37 pts evaluable for best overall response, 6/37 had complete response according to BJP but not to sFLC, interestingly 6/37 progressed after 2-8 months. Twenty-one pts progressed during follow-up: PD was detected only by sFLC in 4, only by BJP in 1. Both tests were able to detect PD in 16 pts: at the same time in 5, with sFLC-PD occurring earlier in 7 and BJP-PD occurring earlier in 4 pts.

Summary/Conclusions: Both sFLC and BJP measurement are useful in LCM pts for disease monitoring, however sFLC assessment appears to be more sensitive in MM and early relapse identification. These data suggest that BJP could be substituted by sFLC assessment in LCM. In our series only 1 case showed BJP-PD according to IMWG occurring earlier than sFLC- PD but was considered not clinically significant. On the contrary 5 pts in BjP-6 had “measurable disease” only by sFLC without having reached FLC-GR. Limits of our study are a small number of pts, inhomogeneous duration of thera- py and follow-up and retrospective analysis.

E1271
SUPPRESSION OF THE NON-MONOCOALANAR AS NEW BIOMARKER FOR DE-NOVO MULTIPLE MYELOMA PATIENTS AT DIAGNOSIS AND AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION
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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 chains 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 made with Prism 6.0.

**Results:** The median OS of the 85 patients was 54% and 26 patients deceased during the study due to MM. The median value of i/u HLC ratio was 80 (31.5-319.7). At diagnosis, a i/u HLC ratio>80 was significantly associated with worse OS (46 vs 61%, p=0,005) and shorter PFS (23 vs 42%, p=0,006). Severe HLC-matched pair suppression (i.e. more than 50% below the lower reference range) was identified in 68% of the newly diagnosed patients and was associated with significantly shorter OS (35 vs 81%, p=0,004) and PFS (21 vs 50%, p=0,013). Severe (>50%) systemic immunoparesis of non-monoclonal immunoglobulins was identified in 64% of the patients at diagnosis and was also significantly associated with shorter OS (32 vs 81%, p=0,030) but not with shorter PFS (26% vs 44%, p=0,306). The evaluation of other clinical variables on patient’s outcome are shown in table (see Table). In multivariable 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, severe HLC-matched pair suppression and albumin were found as independent risk factors 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.

**Summary/Conclusions:** Severe HLC-matched pair suppression and i/u HLC=80 are associated with worse OS and shorter PFS in MM patients suggesting a potential use of these parameters as prognostic biomarkers in newly diagnosed patients. Severe HLC-matched pair suppression is an independent risk factor for OS whereas i/u HLC>80 is independently associated with shorter PFS. In patients after ASCT, severe HLC-matched pair suppression reflects the persistence of clonal cells that is not associated with severe systemic immunoparesis.

**E1272**

**SURVIVAL STRATIFICATION OF PATIENTS WITH MULTIPLE MYELOMA (MM) AFTER FIRST RELAPSE: SENSITIVITY ANALYSES OF A NOVEL RISK STRATIFICATION ALGORITHM (RSA)**

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**Background:** Established risk stratification tools in MM, such as the International Staging System (ISS) and the revised ISS, have improved overall survival (OS) estimates by combining the strongest known predictors of survival at diagnosis. There remains, however, a need for tools that use additional data available at relapse to improve risk stratification. We previously used real-world data from the Czech Registry of Monoclonal Gammopathies (RMG) to develop a RSA for estimating risk of death in patients with MM starting second line (2L) treatment. A multiple Cox regression model identified predictors of OS at 2L (Table); hazard ratios (HRs) for each predictor were multiplied to obtain an overall score for each patient. A K-adaptive partitioning for survival (KAPS) algorithm stratified patients into risk groups based on these scores.

**Aims:** To investigate how our RSA is affected by: 1) removing cytogenetic abnormalities (CAs) at diagnosis as an OS predictor, as these are not routinely measured in practice; 2) adding 2L treatment as a predictor, as 2L treatment type is likely to affect OS; 3) changing the number of stratification groups.

**Methods:** The analyses used data for 1418 patients aged ≥18 years who were diagnosed with symptomatic MM between May 2007 and April 2016 and who had reached 2L. The Cox model was re-run for two sensitivity analyses: excluding CAs and adjusting for treatment received at 2L (adding bortezomib or lenalidomide vs other treatments as a predictor). The impact of different numbers of risk groups was assessed using KAPS.

**Table 1.**
E1273

REAL-WORLD DATA ON MULTIPLE MYELOMA: A PROSPECTIVE NATIONAL REGISTRY IN URUGUAY ON 224 NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS FROM 2012-2015

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Background: The Uruguayan National Myeloma Registry is the first observational prospective Uruguay registry designed to document clinical characteristics of newly diagnosed multiple myeloma (MM) at diagnosis and outcomes in a real-world setting. It collects detailed data, regardless of 2L treatment type. CAs at diagnosis is a known OS predictor and, as expected, improves the strength of predictions. The practicalities of measuring CAs should be considered, but these data suggest that physicians should be encouraged to assess CAs at diagnosis; CAs at relapse may also be informative. Further validation of this model is required using other real-world and clinical trial data.

Aims: To document current strategies of clinical characteristics at diagnosis, management, outcomes and treatment adverse effects of non-selected newly diagnosed MM patients in a recent period.

Methods: This registry includes all MM diagnosed from January 2012 in all institutions, nationwide. Smoldering MM are not included. We present the analysis of the first 3 years of data collection. Information was obtained from medical records and patients include 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-secretor 2.2% and IgM <1%. Most patients had advanced disease: 79.6% Durie-Salmon stage III (176/221), 48.6% ISS 3 (86/177). Anemia (hemoglobin ≤ 10 g/dl) was present in 48.3%. Osteolytic lesions in 69%, renal impairment (creatinine≥2mg/dl) in 29.5% and hypercalcaemia in 10%. Cytogenetics was evaluated in 150 patients; high risk features were detected in 6.3% by conventional cytogenetics and 19% by fluorescence in situ hybridization. First-line treatment included at least one of the new drugs (Thalidomide, Bortezomib or Lenalidomide) in 92% of patients ≤70 years and in 50% of >70 years. First-line response was available in 73%. Overall response rate (≥PR) was 82.3%, VGPR= 23.2% and CR=15.2%; 9.8% patients achieved stable disease and 7.9% were refractory. Further validation of this model is required using other real-world and clinical trial data.

Reference

E1274

REPRESENTATION OF MINORITIES, THE ELDERLY AND WOMEN IN MULTIPLE MYELOMA CLINICAL TRIALS

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Background: Multiple myeloma (MM) accounts for approximately 1% of all cancers and 10% of hematologic malignancies in the United States (US). MM occurs in all races but the incidence in African Americans is two to three times higher than in non-Hispanic whites. Many clinical trials (CT) lack appropriate representation of specific patients populations, limiting the generalizability of the evidence obtained. We describe the characteristics of CT in MM.

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 (10%) by NCI and 52 (78%) were investigator initiated. Participation in CT varied significantly across ethnic groups, non-Hispanic Whites (NHW) were more likely to be enrolled in CT (EF of 0.23) than African Americans (AA) (EF of 0.08, p <0.0001) and Hispanics (His) (EF of 0.05, p <0.0001). Males had
a higher recruitment rate than females (58% vs 42%), but this could be explained by the higher incidence of MM in this subgroup. Enrollee’s median age was 62 years. Younger pts (<65 years) were more likely to be enrolled in CT than the elderly (66% vs 34%, p<0.0001). Industry sponsored trials were less likely to recruit AA compared with investigator initiated trials (7.6% vs 12%, p=0.0001).

Table 1.

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>2013 MM Prevalence, %</th>
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<tr>
<td>N/A</td>
<td>130 (13)</td>
</tr>
<tr>
<td>Native American</td>
<td>8 (8)</td>
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<tr>
<td>Other</td>
<td>2 (2)</td>
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<tr>
<td>White</td>
<td>931 (931)</td>
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<tr>
<td>Other</td>
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</table>

Summary/Conclusions: Despite the higher incidence of MM in African Americans and the elderly, the former only represented 8.6% of the study participants and 66% of these were less than 65 years of age, perhaps lacking data in the tolerability of these new agents in our aging MM population. We also observed industry studies were less likely to recruit AA patients. Future trials should take extra measures to recruit participants that adequately represent the United States MM population.

E1275

EVALUATION OF TREATMENT WITH NEUROPATHELY IN MULTIPLE MYELOMA AND ITS INFLUENCE ON PHYSICAL AND ROLE FUNCTIONING B. Sidi Mohamed El Amine1,*, H. Asma1, O. Fouza1, S. A. Najet1, Z. Zahia1 1Hematology department, University hospital of Sidi Bel Abbes, Sidi Bel Abbes, Algeria

Background: Peripheral neuropathy (PN) is a major dose limiting and potentially disabling adverse event of commonly therapeutic drugs used in the management of multiple myeloma (MM), including the immunomodulatory drugs (IMIDS, Thalidomide and Lenalidomide), and the proteasome inhibitor (Bortezomib).

Aims: The aims of this study were to (1) perform a psychometric evaluation of PN and (2) examine the prevalence of this complication and its influence on physical and role functioning of MM patients.

Methods: The FACT/GOG-Neurotoxicity (Ntx) subscale for assessing treatment induced PN was evaluated. The 11-item of this questionnaire was administered to patients with MM treated with IMIDS and/or Bortezomib. The subscale was evaluated in 32 patients for internal reliability, construct validity, criteria validity, and compared to NCI grading adverse events (CTCAE version 3). Spearman rank correlation was calculated to determine the impact of PN on functional, physical and role functioning of MM patients, assessed by EORTC quality of life scale (EORTC QLG-C30). A Cronbach coefficient ≥ 0.8 is good. Spearman rank correlation is significant if p < 0.05 or r > 0.5.

Results: Cronbach alpha coefficient for internal consistency of FACT/GOG-Ntx subscale was 0.92, and its correlation with the full CTCAE scale as follows: P=0.0001. All the 11 items exhibited high correlations with the NTX subscale score (r= 0.65-0.79), and the Construct validity of NTX was good. According to FACT/GOG-NTX and NCI-CTCAE, 24 (75%) patients presented PN secondary to IMID or Bortezomib. The PN was severe in 14 (43, 7%) patients, especially those who received Bortezomib associated with IMIDS (71, 4%). PN did not influence the achievement of a very good response of MM to therapy neither a complete remission (P=0.6), but patients with high scores of NTX subscale have reduced functional activities, especially physical and role functioning. The 11-item FACT/GOG-NTx subscale reliably and validly assesses Bortezomib/IMIDS induced PN. This complication is frequent and can alter the functional abilities of MM patients.

E1277

ANALYSIS OF THE CONNECT MM REGISTRY: TREATMENT OUTCOMES AND HEALTHCARE RESOURCE UTILIZATION IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA WHO RECEIVED LENALIDOMIDE MAINTENANCE OR NO MAINTENANCE R.M. Rifkin1, S. Jagnanath2, B.G. Durie3, J.J. Shah4, M. Narang5, H.R. Terebelo6, C.J. Gasparetto7, K. Toomey8, J.W. Hardin9, L. Wagner10, K. Parikh11, S. Abouzaid11, S. Srivinasan11, A. Kitali11, F. Zafar11, R. Abonour12 1US Oncology Research, Rocky Mountain Cancer Centers, Denver, 2Mount Sinai Hospital, New York, 3Cedars-Sinai Samuel Oschin Cancer Center, Los Angeles, 4MD Anderson Cancer Center, Houston, 5US Oncology Research, Maryland Oncology Hematology, Columbia, 6Providence Cancer Institute, Southfield, 7Duke University Medical Center, Durham, 8Steepchase Cancer Center, Somerville, 9University of South Carolina, Columbia, 10Wake Forest University School of Medicine, Winston-Salem, 11Celgene Corporation, Summit, 12Indiana University Simon Cancer Center, Indianapolis, United States

Background: Maintenance therapy post autologous stem cell transplant (ASCT) has been shown to improve clinical outcomes, including time to progression, progression-free survival (PFS), and overall survival (OS) in patients with newly diagnosed multiple myeloma (NDMM) (Sonneveld, 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 treatment on clinical outcomes and HRU in a largely community setting.

Methods: Patients with NDMM who were eligible for enrollment in the registry within 60 days of diagnosis. Patients who completed induction and single ASCT without subsequent consolidation and received lenalidomide (LEN)-only or no maintenance were included in the analysis. HRU (hospitalization rates and length of stay, surgery/procedures, concomitant medications including growth factor, bisphosphonate, and neuropathic pain medication) was assessed from 100 days post-ASCT to the end of years 1 and 2. Data cutoff was Jan 7, 2016 and the median follow-up was 39.3 months.

Results: A total of 1493 patients with NDMM were enrolled in Cohort 1 from Sep 2009 to Dec 2011: 421 patients met the analysis criteria stipulated above. Of these, 108 did not receive subsequent consolidation and were not included in the analysis. A total of 1385 patients (99%) were included in the analysis. Median age at diagnosis was 60 y (range, 24-78); 60% were men, and 86% were white. Baseline patient characteristics except serum haematologica | 2017; 102(s2) | 523
CREATIVE: For patients with NDMM, LEN-only maintenance significantly improved PFS and OS vs no maintenance with no apparent impact on HRU.

E1278

SERUM-FREE LIGHT-CHAINS (SFLC) INSTEAD OF URINE PROTEIN ELECTROPHORESIS (UPEP) FOR MONITORING MULTIPLE MYELOMA (LCMM)


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Background: Response and follow-up criteria in multiple myeloma (MM) are still based on the protein electrophoretic (PEP) quantification of the monoclonal protein (MP) in serum (s) and/or urine (u). Monitoring MP by urine (u) PEP has a very low sensitivity for evaluating variations of small amounts of MP. Since 2001, serum free light-chain assays (sFLC) are available, with demonstrated clinical utility. Dejori et al. have recently reported the usefulness of sFLC for evaluating response in LCMM.

Aims: In this work, we try to validate the use of sFLC assay in the context of GEM/PETHEMA clinical trials in order to evaluate the responses and its advantages in comparison to standard quantification of MP by PEP in serum (s) and/or urine (u). Monitoring MP by urine (u) PEP has a very low sensitivity for evaluating variations of small amounts of MP. Since 2001, serum free light-chain assays (sFLC) are available, with demonstrated clinical utility. Dejori et al. have recently reported the usefulness of sFLC for evaluating response in LCMM.

Methods: We included 169 patients with Bence Jones (BJ) MM with measurable urine disease who have been treated according to GEM/PETHEMA clinical trials (GEM05menos65, GEM05MAS65, GEM2010MAS65 and GEM2012 menos65). Serum FLC assays (Freelite®, The Binding Site, Birmingham, UK) were performed on an automated nephelometer (BNII, Dade Behring / Siemens, Marburg, Germany). The electrophoretic study of the monoclonal component (CM) was performed by capillary electrophoresis (V8, Helena Biosciences Europe), and immunofixation was performed for the Ig, κ and λ chains (SAS-3 and SAS-4, Helena Biosciences Europe).

Results: From a total of 168 patients with measurable urine disease (168 patients with BJ MM and 163 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 isFLC) and we observed a low correlation (Pearson’s r = 0.293, p = 0.003), that should be partly explained by the low profitability and subjectivity of the electrophoresis technique for quantifying para-protein in urine. Figure 1A. The concordance between the classification of the response by uPEP / immunofixation (IF) and by FLCs (Kappa Index=0.425 vs 0.001) is noted in the Table. The normalization of the sFLC ratio (r) <0.0101) was achieved in 35/98 (36%) patients after treatment, associated to a lower risk of progression (normal vs abnormal sFLCr: PFS 60 vs 39 months, p = 0.038) but without impact in overall survival in our series. We also observed that an absolute value of isFLC greater than 50mg/L after treatment was associated with an increased risk of progression, regardless of the response achieved (PFS 60 vs 28 months, p<0.0001). [Figure 1B].

Figure 1.

Summary/Conclusions: There is an acceptable agreement between both methods for response evaluation. The sFLC assays provide a greater sensitivity than the urine protein electrophoresis for monitoring low levels of disease in certain cases with measurable disease at diagnosis (isFLC ≥100) being useful for its follow-up, and also provide prognostic value as a predictor of progression.

E1279

TOPSPIN: A NOVEL ALGORITHM TO PREDICT TREATMENT SPECIFIC SURVIVAL IN CANCER

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Background: In recent years many novel treatments have been introduced for Multiple Myeloma (MM), leading to an improved survival. However, this has also led to the situation where many different treatment combinations are used, without a clear indication which patient will benefit most from which treatment. It is increasingly recognized that genetic heterogeneity between tumors influence treatment response. Patient outcomes may be improved by selecting the right treatment for the right patient at the moment of diagnosis. This requires the discovery of predictive markers, for example gene expression signatures, that can aid in this treatment decision. Here we present TOPSPIN (Treatment Outcome Prediction using Similarity between PatientNts), 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 genetically similar patients who received a different treatment should have a large difference in survival, given that genetic similarity is defined in a manner that is relevant to treatment response. This principle is used to identify prototype patients: patients who received the treatment of interest and have a larger than expected survival difference with the genetically most similar patients who received another treatment. Genetic similarity is defined separately for 10 581 gene sets based on Gene Ontology (GO) annotation. These prototype patients are used to define a classifier: new samples who exhibit a similar gene expression profile to the prototype patients will benefit more from the treatment of interest. Here we use TOPSPIN to predict which patients will benefit from the proteasome inhibitor bortezomib. We combine tumor gene expression data from the Total Therapy 2, Total Therapy 3 and HOVON-65/GMMG–HD4 phase III clinical trials into one dataset comprising 910 patients, split into a bortezomib arm (n=407) and a non-bortezomib arm (n=503). Progression free survival is used as outcome measure. This dataset was split in a training set (n=606) and a test set (n=304). The test set is not used at any point in the training procedure and is only used for independent validation.
Results: We successfully identify gene sets that enable us to predict which patients will benefit most from bortezomib. The top 8 performing GO sets based on Hazard Ratio (HR) were combined to achieve the final classification. In the training set 28.4% of patients were 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.

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 treatments 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] heart). 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 cardiologists and hematologists (Table 1). Caregivers echoed the multitude of distinct physicians visited before diagnosis (Table 1). Patients reported that biopsy of fat pad, kidney, or heart was the predominant diagnostic test performed (Table 1). Hospitalization was prevalent; 55.5% (n=76) patients reported amyloid-related cardiac hospitalization. Moreover, 31.3% (n=43) of patients reported the need for air travel for physician consultation.

Table 1.

Summary/Conclusions: This represents the first survey compiling both caregiver and patient experiences with AL amyloidosis. Alignment of caregiver with patient responses validates our patient-directed research. Patients with AL cardiac amyloidosis frequently receive misdiagnoses and sometimes receive incorrect treatment for the misdiagnosed condition. Disease awareness among all specialists is vital, especially among those to whom patients are initially referred due to the nature of their initial symptoms.
imunomodulatory agent (IMiD)–free regimens. Analysis using a fixed-effects model found that DRd compared with other IMiD-containing regimens in Network 1, and DVD compared with other IMiD-free regimens in Network 2 produced PFS and OS among patients with MM. **Results:** 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 TM of MM. **Aims:** The study aimed to describe trends in demographics, TX patterns, and sequencing for newly diagnosed MM (ndMM) patients (Pts) in the United States (US) using an enhanced Electronic Health Records (EHR) database. **Methods:** A retrospective observational study of ndMM Pts was conducted utilizing EHR from a nationally-representative database (Flatiron Health). The Flatiron MM provider network comprises over 260 clinics throughout the US. Pts with an ICD-9 (293.0x) 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) TX therapy was defined as the 1st episode of an eligible systemic Tx given after or up to 14 days before the index date. Regimen 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 (lg) classes at diagnosis were IgG (51.8%) and IgA (18.9%). Median follow-up time for these groups was 471, 730, 928, 1132, and 610 days respectively. Among treated Pts, 205 (12.7%), 208 (28.2%), 109 (33.5%), and 98 (38.1%) received at least 1 stem cell transplant (SCT), respectively. Of Pts receiving 1L TX, 984 (33.6%) received IMiD compound +PI, 715 (24.9%) received IMiD compound therapy in 1L. The use of IMiD compounds +PI in 1L increased during the study period for SCT and non-SCT Pts (NSCT) from 40.6% and 21.5% in 2011, to 66.7% and 46.8% in Pts diagnosed in 2016. In Pts who received a SCT (n=618), the most common 1L regimens were lenalidomide + bortezomib + dexamethasone (RX; n=217, 43.9%), cyclophosphamide + bortezomib + d (CX; n=124, 24.1%), lenalidomide + d (RD; n=70, 11.3%), and bortezomib + d (VD; n=57, 9.2%). In NSCT Pts (n=2307), the most common 1L regimens were RVD (n=642, 27.8%), Vd (n=510, 22.1%), and Rd (n=412, 17.9%). Among the RvD NSCT Pts with a documented 2L (n=189), the most common 2L regimens were CyBord (13.2%), carfilzomib monotherapy (7.4%), pomalidomide + d (6.9%), and lenalidomide + d (6.9%). Documented death occurred in 785 (23.3%) Pts during the study period. **Summary/Conclusions:** Over time, RX has become the most common 1L regimen for SCT and NSCT Pts with MM after a median follow up of 15.9 months, many patients remain in the initial LOT. With a longer follow up, we will be able to observe sequencing and patterns of treatment in later LOTs.
Results: At a median follow-up of 9.8 months in a protocol-specified interim analysis, 67 patients had disease progression or death were observed (in 18 of 66 patients (27.2%) in the group B vs 28 of 68 (41.1%) in the control group (p<0.001)). A significantly higher rate of overall response was observed in the group B than in the group A (68.7% vs 62.9%, p<0.001), as was a higher rate of complete response or better (39.2% vs 16.1%, p<0.001). The most common adverse events during the treatment was myelotoxicity (neutropenia in 68.6% of the patients in the therapy group B vs 42.1% of those in the control group A), anemia (in 21.5% vs 13.6%) and thrombocytopenia (in 13.8% vs 8.7%).

Summary/Conclusions: In patients with relapsed multiple myeloma, the addition of daratumumab to lenalidomide and dexamethasone appeared active and resulted in significantly improved progression-free survival. However it was associated with a higher risk of myelotoxicity.

E1285
COMPARISON BETWEEN IMMUNOFIXATION NEGATIVITY AND NORMAL FREE LIGHT CHAIN RATIO WITH MULTICOLOUR FLOW CYTOMETRY FOR RESPONSE ASSESSMENT IN PATIENTS WITH MULTIPLE MYELOMA WITH VGPR OR BETTER
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Background: Urine and serum Immunofixation electrophoresis (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)/oligosacryl- 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 forty-four complete IFE, FLC, and MFC data set of 126 patients (LCMM/OSMM 40, IIMM 86) with ≥VGPR were analysed. Normal FLC at VGPR, cCR and MRD- CR was 65.0%, 78.4% and 76.8% in IIMM, and 0%, 21.4% and 100%, respectively, in LCMM/OSMM. The percentages of sample at MRD levels of MRD >10^-3, 10^-3 ≤MRD >10^-4 and 10^-4 ≤MRD in LCMM/OSMM were significantly higher than in IIMM (12.5%, 50.0%, and 100% for negative uIFE, and 0%, 11.5% and 100% for normal rFLC, respectively. These figures in IIMM were 23.0%, 41.6%, 81.4% for negative sIFE, and 53.8%, 75.0% and 88.8% for normal rFLC, respectively. Positive/negative predictive value (PPV/NPV) of uIFE and rFLC for MRD in LCMM/OSMM was 100%/94.8% and 100%/85.0%, respectively, while those were 90.6%/45.8% and 88%/93.4% in IIMM, respectively.

Summary/Conclusions: Our observations confirmed that FLC test has greater sensitivity than uIFE for detection of the monoclonal component, and that normalization of sFLC ratio is highly predictive of MRD negativity in patients with LCMM/OSMM. The proportion of negative sIFE samples increased with depth of MRD, but the FLC response did not appear to parallel with the depth of response in IIMM. We recommend that FLC test should be incorporated into response assessment in LCMM/OSMM as an alternative to 24-h uIFE, and both negative sIFE and normal rFLC are still useful for response assessment of residual clonal PCs in IIMM.

E1286
DARATUMUMAB IS AN EFFECTIVE AND SAFE SAVAGE THERAPY IN RELAPSED/REFRACTORY PATIENTS WITH MULTIPLE MYELOMA AFTER AUTO-ALLILOGENEIC STEM CELL TRANSPLANTATION
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Background: Daratumumab is a human monoclonal antibody that targets CD38, a cell surface protein that is overexpressed on multiple myeloma cells. The drug became the first monoclonal antibody as single agent therapy approved by the FDA for the treatment of multiple myeloma. The role of allo-SCT in myeloma patients (pts) remains unclear; nevertheless, the registry study of EBMT suggests an increasing rate of allografts in Europe in last years. Despite the potentially curative potential of this approach, the increased relapse rate and low PFS remain a central clinical problem.

Aims: In this single center retrospective analysis, we report on our experience with the use of daratumumab to lenalidomide and dexamethasone as salvage therapy. Before allografting 9 pts received at least 1 salvage therapy line prior to the allo-SCT. The allografts were performed from unrelated donors (MUD, n=9; MMUD, n=4) or matched related donors (MRD, n=3). The median number of salvage lines post-transplant and prior to first daratumumab infusion was 3 (1- 4).

Results: The median number of infusions was 13 (3-22). A total of 16 and 15 pts were available to safety and efficacy evaluation, respectively. The safety was assessed according to the Common Toxicity Criteria (CTC). A total of 20 adverse events were observed in 16 pts: dyspnea (CTC1, n=3; CTC2, n=1), bronchospasm (CTC2, n=2) shivering (CTC1, n=3), cough (CTC1, n=1; CTC2, n=1), musculoskeletal pain (CTC1, n=4), acute coronary syndrome (CTC3, n=1), skin rush (CTC2, n=1), pressure on eyes (n=1). Two patients developed late onset infections (pneumonia and infection of urinary tract) followed by temporarily therapy interruption. We observed a decrease of Tregs (CD4+CD25highFoxP3+) 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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Results: 482 pts were eligible, 343 (71%) were W, 52 (10%) H, 50 (10%) B, 19 (3%) A, and 18 (3%) O. Median age was 65 years, 54% were male, and 28% ISS stage 3. There were no statistically significant differences in FISH abnormalities between the M. Overall W had more abnormalities in IGH r (t(4;14), t(11;14), t(14;20), 1q21 gain compared to M. Most notably W had more IGH r (39% vs 28%, p=0.019) and t(11;14) (20% vs 12%; p=0.024). There were no statistically significant differences between W and M in the high risk FISH abnormalities.

Table 1.

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$p$-value compares W vs M. * means statistical significant ($p$-value<0.05)

Summary/Conclusions: W had significant differences in FISH compared to M. W had more IGH r and t(11;14) than M, and there was no difference in high risk FISH abnormalities between W and M. This study confirms the biological racial disparities that exist in minorities with MM. Further studies with more inclusion of minorities are needed to elucidate these disparities and its effects on risk stratification and outcomes.

E1289

POMALIDOMIDE ALONE OR IN COMBINATION WITH LOW DOSE DEXAMETHASONE AS MAINTENANCE FOLLOWING INDUCTION WITH POMALIDOMIDE AND LOW DOSE DEXAMETHASONE IN RELAPSED AND REFRACTORY MYELOMA (ALLG MM14)

Background: Whilst the addition of dexamethasone to upfront therapy with Immunomodulatory (IMiD®) agents is important to mediate rapid reduction in disease burden, preliminary findings suggest that the NK stimulatory effects of IMiD® compounds are best harnessed without the co-administration of dexamethasone, and may be especially effective in the setting of minimal disease burden (in the maintenance setting for example) when some inherent immune recovery has occurred. However this has yet to be confirmed in a prospective clinical trial.

Aims: To evaluate the effect of maintenance with POM alone (Arm 1) versus POM-LoDEX (Arm 2) on progression free survival (PFS), overall survival (OS), and kinetics of response (overall response rate (ORR)) in relapsed myeloma (MM) patients refractory to lenalidomide (L-RLEN) demonstrating stable disease (SD) or better following salvage treatment with pomalidomide (POM) and low dose dexamethasone (LoDEX) induction.

Methods: Multicentre, open-label, randomized phase 2 study of relapsed R-LEN 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 11 sites were enrolled on to the study (M:F 80:74), with a median age of 67 years (range 35-88). Median number of prior lines of therapy was 4.5 (2-14). All patients had failed LEN (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 the LoDEX induction regimen. POM-LoDEX demonstrated a median PFS of 13.7m for POM-LoDEX (Arm 2) versus 5.6 for POM-LoDEX (arm 2) (p=0.39). The PFS hazard rate for Arm 2 was lower (p=0.003) and OS was (p=0.003), irrespective of first line therapy (bortezomib-based regimens for fit patients or alkylating-based treatments for unfit patients). Interestingly, we also observed an increased percentage of CD138+/CD19+ PCs (21%) expressing VEGF in MM patients with a more favorable PFS (p=0.04) and OS (p=0.008).

Summary/Conclusions: The results of our investigation showed that CD138+/CD19- and CD138+/CD19+ PCs have differences in what concerns VEGF expression, not only in MM patients, but also in immunus patients. The increased expression of VEGF in clinical PCs from MM compared tomusGM patients evidences the relevance of VEGF in myelomagenesis. We also demonstrated a negative prognostic impact of an increased VEGF expression in CD138+/CD19- PCs, highlighting the role of VEGF in the survival and maintenance of clinical PCs and as a predictor of outcome in MM progression. The association between the percentage of CD138+/CD19+ PCs and survival supports the idea that these cells may not be neutral players in the complex pathogenesis of MM. The results of our study should be further investigated in larger series of patients.

E1288

RACIAL DIFFERENCES OF FISH ABNORMALITIES IN MINORITIES WITH MULTIPLE MYELOMA: A SINGLE-CENTER EXPERIENCE

Methods:

- We included 128 patients with monoclonal gammapathies, 60 patients with newly diagnosed symptomatic MM and 68 with monoclonal gammapathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (CN). t(4;14) and t(11;14) were performed to assess the VEGF and VEGFR accuracy as diagnostic and prognostic biomarkers.
- All M (Hispanic (H), Black (B), Asian (A) and Other (O)) were included into the same group for statistical analysis.
- Due to smaller numbers, all M (Hispanic (H), Black (B), Asian (A) and Other (O)) were included into the same group for statistical analysis.

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 enhanced 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 marked heterogeneity in the expression of VEGF between plasma cells (PCs) from the same MM patient. However, no clear association with expression levels, phenotypic subtypes of PCs and prognosis was demonstrated.

Aims: The present study aimed to evaluate the expression levels of VEGF and VEGF receptor (VEGFR) on phenotypic subtypes of PCs in patients with monoclonal gammapathies and to explore its role as diagnostic and prognostic biomarkers.

Methods: We include 128 patients with monoclonal gammapathies, 60 patients with newly diagnosed symptomatic MM and 68 with monoclonal gammapathy of uncertain significance (MGUS) and also from 11 non-neoplastic controls (CN). (t(4;14), t(11;14), t(14;20), t(4;14), t(14;16), del13q, del 17p, 1q21. Chi-square was used for statistical analysis. Due to smaller numbers, all M (Hispanic (H), Black (B), Asian (A) and Other (O)) were included into the same group for statistical analysis.
POMALIDOMID IS MORE EFFECTIVE IN REAL CLINICAL PRACTICE THAN IN RANDOMIZED TRIAL – AN OBSERVATIONAL STUDY OF THE CZECH MYELOMA GROUP

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Background: The combination of pomalidomide and low-dose dexamethasone (Pom-Dex) is a perspective option for patients with end-stage relapsed/refractory multiple myeloma (R/RMM). 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 R/RMM 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.

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 thrombocytenia 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 R/RMM 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.

E1290

RAD REGIMEN AS INDUCTION BEFORE ASCT: OUTCOMES, SAFETY AND EFFECTS ON BONE METABOLISM AND ANGIOGENESIS; FINAL RESULTS OF A PHASE 2 STUDY OF THE GREEK MYELOMA STUDY GROUP

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Background: There is limited published data on the efficacy and safety of the combination of lenalidomide, adriamycin and dexamethasone (RAD) as induction therapy for newly-diagnosed myeloma (NDMM) patients who are eligible for autologous transplantation (ASCT).

Aims: The primary endpoint of this phase 2 study was the assessment of overall response rate (ORR) after 4 cycles of RAD induction in NDMM patients eligible for ASCT. Secondary endpoints included safety, progression-free survival (PFS), time to progression (TTP) and overall survival (OS). Exploratory endpoints included: i) the yield of stem cell collection after RAD; ii) the effects of RAD on biochemical markers of bone metabolism: CTX, TRACP5b, bone-alkaline phosphate (bALP), P1NP, osteocalcin, soluble RANKL, osteoprotegerin (OPG); iii) the effect of RAD on angiogenic cytokines: angiopep-1 (Ang-1) & -2, angionatin (Ang), VEGF and bFGF.

Methods: Lenalidomide was administered at a dose of 25mg, po, daily, on days 1-21 of a 28-day cycle; dexamethasone was given at a dose of 40mg, po, on odd days (1, 8, 15, and 22) while adriamycin was administered as IV bolus infusion at a dose of 9mg/m2, on days 1-4 of each cycle. Serum levels of the above markers of bone remodeling and angiogenesis were measured before and after 4 cycles of RAD, using ELISA methodology.

Results: Between November 2014 and February 2016, 45 patients (median age 59 years) were enrolled. Osteolytic lesions were present in 33 (73%) patients, while 3 (6.6%) had hypercalcemia (>11mg/dl). All but one patient completed 4 cycles of RAD. Best response included one (2.2%) CR, 8 (17.8%) VGPRs, 21 (46.7%) PRs, for an ORR of 66.7%, while 14 (31%) patients had
stable disease and one progressed during the 4th cycle of treatment. After ASCT the ORR was 84.4%: 6 (13.3%) patients achieved CR, 13 (28.9%) VGPR and 19 (42.2%) PR. Adverse events of grade 3 or 4 included mainly anemia (4 patients), neutropenia (3, 6.6%) and febrile neutropenia (one patient). After a median follow-up of 1.1 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-RAD induction (mean±SD: 8.94±6.50 x10⁶/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 AIOLOS/Ang-2, bALP and P1NP compared to healthy subjects of similar age and gender (p<0.01 for all comparisons). RAD therapy resulted in a reduction of circulating CTX (p=0.03), TRACP-5b (p=0.01), Ang (p=0.02), VEGF (p=0.01) and bFGF (p=0.01). Moreover, RAD increased serum levels of bALP (p=0.036), 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, with median PFS and OS not reached after 104.4 months from initiation of therapy (p=0.02). Time to next treatment (TTNT) in patients receiving ASCT or not was 30.1 months (5-122.7) vs 10.3 months (0.7-70.5) from 1st to 2nd line tx and 12 months (0.3-121.9) vs 6.3 months (1.4-61.6) from 2nd to 3rd line tx (p=0.028). The early mortality (within the first year) was 5.9% (3/52) 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 (21.7% vs 9 (6.2%), p=0.006).

New drugs toxicity: with Bor-based tx 30% of pts complaint neurological, 20% gastrointestinal and 18.2% hematologic toxicity; with Len-based tx 36.4% infective events and 28.9% hematologic toxicity. Median OS in pts st 65 yrs was 7 vs 4.8 years (p=0.001), of note considering pts st 65 yrs treated before 2007 median OS was 5.5 vs 3.1 yrs (p=0.001) and after 2007 median OS was not reached vs 7.5 yrs (p=0.034).

Summary/Conclusions: Our real life data show how MM therapeutic scenario have changed during the last 15 yrs. The tremendous improvement obtained 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.
Summary/Conclusions: In conclusion, our results suggest that CUL4A expres-
sion could serve as prognostic marker for patients assigned to IMiDs containing
regimens. Further analysis of the expression of other E3 ligase complex pro-
teins in a larger patient cohort is in progress.

Acknowledgement: This work is funded by a grant No 2014/13/B/NZ5/03160
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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 Sur-
vival. It remains unclear whereas these patients should all receive maintenance
therapy with it’s toxicity and cost.

Aims: To assess efficacy of maintenance therapy with Bortezomib in patients with
MM, who have achieved complete remission after ASCT with MRD positive
and negative status.

Methods: From January 2014 to February 2016 52 patients with MM (19 male
and 33 female) ages from 24 to 66 years (median 54 years) who have achieved
complete remission after ASCT were randomized for a year-long maintenance
therapy with Bortezomib. On 100th day after ASCT and after completion of
maintenance therapy samples bone marrow from all patients were assessed
using 6-color Flow Cytometry to detect MRD. We chose Relapse-Free Survival
(RFS) as the indicator of maintenance therapy efficacy. Kaplan-Meier survival
curves were compared using log-rank test. Statistical analysis was performed
using SAS 9.4.

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: 35.5–70.5%) vs 37.2% (95% CI: 25.4–49.3%). The MRD-positivity
significantly increases the risk of relapse (HR=1.7; 95% CI: 1.2–3.4; p=0.05).
Two year cumulative probability of relapse after ASCT in patients with MRD-
negative status, who had (n=15) and hadn’t received (n=10) maintenance ther-
apy 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 (sin-
gle and tandem) in a single centre, with 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 raising 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 con-
ditioning, 32 in combination with total body irradiation. Tandem ASCT (defined
as second transplantation performed within 6 months after the first) was per-
fomed 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 trans-
plantation resulted in superior OS (203 vs 86 vs 68 months respectively,
p<0.0001) and PFS (60 vs 38. vs 25 months respectively, p<0.0001) (figure).
Thirteen percent of patients who underwent tandem ASCT are alive and pro-
gression-free more than 10 years after the procedure. Fourteen patients devel-
opped secondary malignancies.

Figure 1.

Summary/Conclusions: Our results suggest that tandem ASCT is a very effec-
tive treatment modality that can partially substitute for the absence of expensive
novel agents with 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 pro-
longed PFS.

E1297
EXTRAMEDULLARY DISEASE IN MULTIPLE MYELOMA PATIENTS
UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION:
CLINICAL IMPACT IN DIAGNOSIS, TREATMENT AND OUTCOME
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Background: Extramedullary disease (EMD) is defined as an infiltrate of clonal
plasma cells outside of the bone marrow. The presence of EMD in multiple
myeloma (MM) patients (pts) at diagnosis is a relatively uncommon presentation
and accounts for about 13% (6-20%) of MM pts. Although several studies

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showed an association of EMD with other adverse prognosis factors and unfavourable outcomes, reports evaluating 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, 28% 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 (response/very good partial response [CR/VGPR]) proportions previously to ahSCT (30.4 vs 53.2%; p=0.009), as well as at 100 days after ahSCT (D100) (41.3 vs 59.6%; p=0.037). However, no differences were found concerning refractoriness to first line therapy or proteasome inhibitor (PI) treatment, despite EMD pts received a higher mean number of therapeutic lines previously to ahSCT vs pts without EMD (41.3 vs 28.9; p=0.025). After a median follow-up of 48.6 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 18.9 months; p=0.04), but with no impact in PFS (p=NS), and there were no differences concerning radiotherapy treatment (72%) or thalidomide maintenance after ahSCT (32%) (p=NS).

Summary/Conclusions: In our cohort, EMD prevalence was significantly higher than usually described in the literature. This observation was probably associated with more carefully surveillance of EMD in ahSCT candidates. EMD was associated with a lower proportion of CR/VGPR previous to ahSCT and at D100 evaluation, even after a higher number of therapeutic lines, although we failed to demonstrate that EMD was an independent prognosis factor for PFS and OS. PI seem also to be the best first-line therapeutic approach for EMD pts. In conclusion, our study suggests that EMD is underdiagnosed in MM pts. It is necessary to achieve a better knowledge of the physiopathology of EMD, in order to define better treatment options that may overcome its negative impact in therapeutic response.

E1298
DIFFERENCES IN PATIENT AND DISEASE CHARACTERISTICS OBSERVED AT INITIATION OF FIRST-LINE AND INITIATION OF SECOND-LINE TREATMENT IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA IN THE CZECH REPUBLIC

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Background: Tools such as the International Staging System (ISS) and the revised ISS (R-ISS) help to stratify risk of disease in patients with multiple myeloma (MM), enabling assessment of survival expectations. These tools are based on factors measured at diagnosis only; understanding the role of these factors at relapse is less clear. Patient characteristics change between first-line (1L) and second-line (2L) treatment. Predicting survival using tools that include patient characteristics measured at diagnosis is thus widely used, but less relevant than other tools that consider factors measured at relapse. The Registry of Monoclonal Gammapathies (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 patients who relapse after aHSCT and could contribute to improved predictive tools that can better define survival estimations at first relapse by considering patients’ experiences at 1L.

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 (≥18 years old) initiating 1L treatment for MM between May 2007 and April 2016 were included (N=3027); those with smoldering MM were excluded. Patient and disease characteristics were extracted at initiation of 1L and of 2L treatment. Repeated measurements were available only for those who initiated 1L and 2L treatment (1L+2L group; N=1418); patients who did not start 2L treatment may have been in remission, lost to follow-up or had died. Patients who received 1L+2L treatment, their health status improved between initiation of 1L and 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% vs 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 (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/Conclusion: Patient health was better at initiation of 2L treatment than at initiation of 1L treatment. At relapse, patients are likely to be closely monitored and are able to initiate the next treatment line while in relatively good health; at initiation of 1L, patients may have experienced deterioration in health which could have triggered their diagnosis. These findings illustrate how patient characteristics change at relapse and that the evolving 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 2018, 87 patients with NDMM and measurable disease (serum and/or urine M protein) were treated with BRs. Both patients eligible and non-eligible to ASCT were included in the study; patients undergoing ASCT were censored at the time of transplant. Median age was 66 (range 32-87); males were 51 (59%); 72 (83%) patients were in 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).

**Summary/Conclusions:** Overall, our data demonstrate a significant impact of EGR on PFS in NDMM patients after BRs, irrespective of median age at diagnosis. In presence of high cytogenetic risk EGR is associated with prolonged PFS, although not significantly. Ongoing analysis on larger cohort of high risk patients will confirm the impact of EGR on PFS also in this group of patients. Based on our data kinetic of response, deriving from EGR assessment, may provide information on both disease aggressiveness as well as clinical outcome, thus representing a novel, surrogate marker for an early survival analysis, with favorable cost-effectiveness characteristics. In summary biological and clinical information deriving from EGR analysis combined with cytogenetic risk evaluation and patient-related (age, comorbidities) characteristics, may represent a useful tool to make clinical decisions. Further prospective evaluations are needed to include this marker in clinical practice.

**E1300**

**RELATIVE PROGRESSION-FREE SURVIVAL OVER TIME OF NOVEL TRIPLET REGIMENS FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA**

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**Background:** In combination with lenalidomide (REV/LIMID®) and dexamethasone (d), elotuzumab (EMPILICTI™, E), carfilzomib (KYPROLIS®, K), and ixazomib (NINLARO®, N) were recently approved for the treatment of relapsed/refractory multiple myeloma (RRMM). In randomized controlled trials, all three drugs showed a significant relative reduction in the risk of disease progression or death as compared to patients who received Rd. To date, there have been no head-to-head trials comparing ErD, KrD, and/or NdR.

**Aims:** To describe the time-specific progression-free survival (PFS) based on published Kaplan-Meier PFS curves for ErD, KrD, and NdR relative to Rd.

**Methods:** Individual patient-level data (IPD) were reconstructed from the published Kaplan-Meier PFS curves from the ELOQUEST-2 (ErD), ASPIRE (KrD), and TOURMALINE-MM1 (NdR) 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: \( \frac{PFS(X \text{ triplet regimen}) - PFS(Rd)}{PFS(Rd)} \). Where \( X \) denotes the Kaplan-Meier PFS estimate at time \( t \), and \( t \) denotes E, K, or N, respectively.

**Results:** PFS 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 NdR. At 24 months, the relative PFS benefit was 45.1% for ErD, 34.3% for KrD and 24.1% for NdR. At 36 months, the relative PFS benefit was 39.9% for ErD and 19.1% for KrD. ErD had a higher relative PFS than NdR for almost the entirety of RRMM treatment. At the end of data availability, KrD and NdR 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.

**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 NdR showed initial benefits which faded by the end of data availability.

**E1301**

**POMALIDOMIDE WITH LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: A PROSPECTIVE ANALYSIS IN A POPULATION-BASED REGISTRY**

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**Background:** Patients with relapsed and/or refractory multiple myeloma (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 prospective 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 alkylator therapy and developed progressive disease on their last therapy. This is a prospective analysis of patients registered at the nationwide Netherlands Cancer Registry. Treatment consisted of 4mg pomalidomide, day 1-21, combined with corticosteroids. Treatment was discontinued in case of progressive disease or unacceptable toxicity. Primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS), overall response rate (ORR), toxicity, response per risk group (based on cytogenetics and ISS at initial diagnosis) and response per age group (>65 vs ≥65 years).

**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 NdR 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] 2.3-6.8). 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-Not reached (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.

Figure 1.

Summary/Conclusions: In this analysis the experience in clinical practice of patients with RRMM treated with a pomalidomide-based regimen is reported. These data support results shown in clinical trials. Preliminary data presented here suggest that older patients and patients with a long interval between initial diagnosis and pomalidomide treatment (indicating a less aggressive multiple myeloma) may benefit from this treatment.

E1302

INVOLVED/UNINVOLVED HEAVY/LIGHT CHAIN INDEX CAN PREDICT PROGRESSION IN MULTIPLE MYELOMA PATIENTS AFTER AUTOLOGOUS PERIPHERAL BLOOD STEM TRANSPLANT. PRELIMINARY EXPERIENCE

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Background: High-dose therapy followed by autologous peripheral blood stem transplant (APBSCT) has demonstrated to improve overall survival and progression-free survival with a high complete remission rate in multiple myeloma (MM) patients. However, most patients eventually present progression or relapse (P/R). Detection of P/R is mainly based on a significant increase of monoclonal protein (MC) or free light chains (sFLC). The identification of new biomarkers to early predict P/R might be clinically useful for an anticipated therapy.

Aims: The aim of our study was to evaluate the potential role of the Involved/Uninvolved Heavy/Light Chain index (I/Ui) in this setting.

Methods: We prospectively followed 44 MM transplanted patients: 19 with IgG-kappa isotype, 11 with IgG lambda, 9 with IgA-kappa and 5 with IgA-lambda. They were followed for 29.0±8.1 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) pair quantification, sFLC and total immunoglobulins levels in serial serum samples collected during the follow-up. Involved/uninvolved index (I/Ui) was calculated using the monoclonal chain (Involved) as numerator and the polyclonal chain of the same class (Uninvolved) as denominator. The HLC ratio (HLC) was calculated as IgGk/IgGk or IgAl/IgAl with normal reference ranges established in 1.3-3.7 for IgG and 0.7-2.2 for IgA.

Results: In IgG MM patients, values of I/Ui were significantly increased in pre-relapse compared to basal samples (8.49±4.01 vs 2.23±0.67 p=0.012). By contrast, this index remained stable along follow-up in patients in complete remission (CR) or with a partial response (PR). However, the later showed higher values of I/Ui ratio, suggesting that the presence of an M-component induces immunosuppression of the uninvolved chain in MM patients. Regarding IgA MM, we established a cut-off value of 2.0 for I/Ui that allowed the discrimination of patients at high risk of early progression (values above 2.0) from those in CR, whose levels of I/Ui are always below 2.0 (p=0.02).

Summary/Conclusions: Our results show that HLC-pair measurement could detect progression or relapse and the increase of MC in transplanted MM patients earlier than other methods. Future studies will need to demonstrate the real value of the I/Ui index as a biomarker to anticipate progression in MM patients subjected to APBSCT.

E1303

MULTIPLE MYELOMA IMMUNOPHENOTYPIC REMISSION IS A SIGNIFICANT PREDICTOR OF PROGRESSION FREE SURVIVAL AFTER FIRST AUTOLOGOUS STEM CELL TRANSPLANTATION - PILOT STUDY

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Background: Minimal residual disease in multiple myeloma assessed by multiparameter flow cytometry has become an increasingly important predictor of progression-free survival (PFS).

Aims: Our primary endpoint was to evaluate PFS in myeloma patients after stem cell transplantation who reached immunophenotypic CR (iCR) versus those who have not.

Methods: We prospectively evaluated prognostic importance of minimal residual disease detection by multiparameter flow cytometry (MFC) in multiple
myeloma patients who underwent autologous stem cell transplantation from January 2014 until December 2016. All patients were uniformly treated with bortezomib-based induction therapy followed by high dose chemotherapy (Melphalan 200mg/m²) and autologous stem cell transplantation. Minimal residual disease (MRD) status was determined by 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% (13/55 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 (0.3365) for ICR group.

Summary/Conclusions: Achieving immunophenotypic CR is clearly associated with longer progression free survival compared to conventional CR. Reaching ICR should be a goal of myeloma treatment.

E1304

REGULATION OF NORMAL AND MONOCLONAL IMMUNOGLOBULIN SECRETION BY CYTOKINES (S- SYNDECAN-1, BLYS & TGF-BETA-1) IN PATIENTS WITH IG-SECRETING B-CELL DISORDERS AT PRESENTATION. PROGNOSTIC IMPLICATIONS

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Background: The most common neoplastic lymphoproliferative diseases that secrete paraprotein are multiple myeloma (MM), Waldenstrom's Macroglobulinemia (WM) and chronic lymphocytic leukemia (CLL). The two first entities secrete paraprotein by definition, while serum free light chains (sFLC) are increased in 50% of CLL cases. Microenvironmental factors, such as soluble Syndecan-1 (ssynd-1) and BlyS normally promote lymphoplasmacytic differentiation as well as their secretory activity, whereas, like TGFβ1, 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 and for monitoring patients, while in CLL, sFLC should be a goal of myeloma treatment.

Table 1.

Aims: To determine any possible relationship between the amount of Igs secreted by BM plasma cells and TGFβ1, as well as with disease outcome.

Methods: We studied 269 patients: 105 with MM (79 IgG and 26 IgA, of whom 33%, 31%, and 36% were staged ISS 1, 2 and 3 respectively), 64 suffering from WM (44%, 28%, 28% staged WM-IPSS 1, 2, 3 respectively), and 100 with CLL (67%, 23%, 10% staged Binet 1, 2, 3 respectively). Patients were regularly followed from the last visit or death (median follow up 5 months). sFLC/sFLCR and HLC/HLCR were determined by nephelometry. They were then followed-up from diagnosis to last visit or death (median follow-up time: 63 months). sFLC/sFLCR and HLC/HLCR were determined by nephelometry. By inhibiting both monoclonal and polyclonal Ig, TGFβ1 correlated with MM in both HLC and FL/C ratios and differences. In addition, the aforementioned variables are prognostic with regard to patients’ outcome.

E1305

PATIENTS WITH MULTIPLE MYELOMA (MM) IN LONG TERM COMPLETE REMISSION (LTRC) AFTER AUTLOGOUS TRANSPLANT (APBSCT) EXPRESS A DISTINCTIVE IMMUNE PROFILE WITH POTENTIAL PROGNOSIS VALUE

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Background: A small fraction of patients with MM could be considered potentially cured as long as they remain for more than six years in long term complete remission (LT-MMC). A retrospective study of the immune status of these patients could highlight interesting information.

Aims: Here we present an observational study that evaluates the numbers and phenotype of T- and B-cells subsets in the peripheral blood (PB) of MM-LTCR patients and compares them to healthy subjects (HS).

Methods: After approval by the ethics committee, we selected 13 patients diagnosed with MM, in sCR according to IMWG criteria for at least six years after APBSCT, and 15 healthy adults (HA) of similar ages as a comparative group. Group MM: 7 males and 6 females; median age: 61. Median follow-up in sCR according to APBSCT was 8 years (range 6-19). Group HA: 5 females and 10 males, median age 40 (36-78) Immunophenotype 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 regulatory T-cells, as well as subpopulations of B-cells: naïve, transitional, marginal zone-like, class-switched memory and plasmablasts. In order to confirm their specific immune signature, the analysis was repeated in the same LTRC-MM patients one year after the first analysis was done. A Kruskal-Wallis test was used to evaluate differences among the studied groups. A posteriori test was done to compare the control group with the two patient’s group (patients and patients +1 year), independently of each other. A Wilcoxon matched test was used to compare a patient under group “patients” with the status of the same patient under the second 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 naive CD4+ T-cells (CD27-CCR7+CD45RA+); p=0.0004) and an increment of the effector memory CD4+ T-cells (CD27-CCR7+; p=0.0028), both CD27-CCR7+CD45RA and CD27-CCR7+CD45RA+. Similar results were found within the DB+T-cells. No differences were observed in the T-cells defining CD4+CD25hiCD127. The percentage of total B-cells in the patients was within the normal range and no significant differences were found when compared to HA. However, naive B-cells (CD27+IgD+IgM+) proportion was higher in patients and a corresponding reduction of marginal zone-like B-cells (CD20-CD27+IgD+) and IgM+ (p=0.0047) and class-switched memory B-cells (CD27+IgD-IgM; p=0.0043) was observed. No differences were observed in the percentage of transitional B-cells (CD27-CD10+CD38+) or plasmablasts (CD27+ CD38*) in the PB of the two groups. When the analysis was repeated in the same LTRC-MM patients one year after the first analysis, no changes were detected neither when analysed as a group nor when analysed individually.

Summary/Conclusions: The MM-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, involving plasma cells that might compete with myelomatous cells for normal bone marrow niches. The precise role of these refined immunological studies in the monitoring and therapeutic decisions in MM patients, and also in the duration of sCR, should be defined in the future.

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E1306

IMPACT OF THE AFFORDABILITY OF NOVEL AGENTS IN PATIENTS WITH MULTIPLE MYELOMA: REAL WORLD DATA ON CURRENT CLINICAL PRACTICE IN MEXICO

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E1307

BASAL CALCIUM, AN IMPORTANT ELEMENT IN THE DEVELOPMENT OF CALR MUTANT MPNS

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Background: Calreticulin (CALR) is a calcium (Ca2+) buffering chaperone mutation of which has recently been associated with essential thrombocythemia and primary myelofibrosis without JAK2 mutations. These mutations have been suggested to impair the Ca2+buffering activity of Calreticulin due to a change of the negative charge in its C-terminal domain. Ca2+ is known to be important during megakaryocyte activity; however its role during megakaryopoiesis and the possible link of CALR mutations and abnormal megakaryocyte production due to impaired Ca2+ buffering activity in myeloproliferative neoplasms (MPNs) remains unclear.

Aims: Here we aim to understand how basal Ca2+ fluctuates during normal megakaryopoiesis and how CALR mutations could affect the basal Ca2+ levels in megakaryocytes in MPNs.

Methods: Ca2+ staining was performed using Flu-8 dye and Ca2+ basal levels were measured by flow cytometry. Changes in basal Ca2+ during megakaryopoiesis using two cellular systems, K-562 cells and mouse bone marrow cells, were measured each 24 hours. Further studies using CALR mutant cellular models were performed using the same methodology.

Results: Our results showed a characteristic behaviour of fluctuations of basal Ca2+ in normal megakaryopoiesis. Changes in basal Ca2+ during megakaryopoiesis, where Ca2+levels decrease in the last stage of megakaryocyte formation. These results suggest that Ca2+ reduction could be essential for megakaryopoiesis. In order to understand how CALR mutations affect basal Ca2+, Marimo cells and Dami cells expressing CALR mutations were analysed. Here we show a decrease in basal Ca2+ in Marimo cells and Dami-CALR type 2 mutation compared to the controls. Moreover, Dami-CALR type 1 did not show any significant reduction, suggesting possible differences in Ca2+ behaviour depending in CALR type mutation. We are currently working in the analysis of basal Ca2+ fluctuations during megakaryopoiesis in the presence of CALR mutations and preliminary results show abnormal basal Ca2+ levels throughout all the process of megakaryocyte differentiation.

Summary/Conclusions: Altogether, our findings indicate that basal Ca2+ could be an important element during megakaryopoiesis and CALR mutations found in MPN could impair the normal production of megakaryocytes due to changes in cellular Ca2+. However, further analysis need to be done in order to understand the role CALR mutations and their effect in the Ca2+ buffering activity of CALR in MPNs.

E1308

THE INHIBITION OF JAK/STAT SIGNALING IS COMPENSATED BY ACTIVATION OF MAPK PATHWAY IN MYELOPROLIFERATIVE NEOPLASMS

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Background: Myeloproliferative neoplasms (MPN) remain incurable regardless of advancement in the use of JAK2 inhibitor Ruxolitinib, which competence is unrelated to the JAK2V617F mutation.

Aims: We want to explore JAK2 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. Hexam bromocyclohexane 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 the constitutive activation of JAK/STAT3 signaling in HEL cells as well as in granulocytes of JAK2V617F positive ET and PMF. Absence of JAK2V617 mutation supported dephosphorylation of JAK/STAT3 pathway by JAK1/2 inhibitors in ET, but not in PMF. JAK1/2 inhibitor
Ruxolitinib largely activates MAPK signaling in MPN, while slightly PI3K/AKT signaling in PV and JAK2V617F negative PMF. Specific JAK2 inhibitor Hexa-bromocyclohexane activates PI3K/AKT signaling in JAK2V617F positive ET, but reduced in JAK2V617F negative ET and PMF.

Summary/Conclusions: This observation support cross-talk between examined pathways, where inhibition of JAK/STAT3 signaling is compensated by activation of MAPK pathway irrespective of JAK2V617F mutation, while PI3K/AKT signaling demonstrates JAK2V617F dependence in MPN.

E1309
CIRCULATING PLATELET AND MEGAKARYOCYTE-DERIVED MICROPARTICLES OF JAK2V617F MUTATED PATIENTS WITH MYELOFIBROSIS ARE DISRUPTED: A NOVEL LIQUID BIOPSY TOOL OF RESPONSE TO RUXOLITINIB?

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Background: Microparticles (MPs) are small vesicles (0.1-1 micron) deriving from plasma membrane budding during homeostasis and cell activation. MPs express antigens and contain constituents from cell of origin and are increased in conditions that are characterized by high cell turnover or death, particularly inflammatory, autoimmune and neoplastic diseases. Myelofibrosis (MF) is a clonal neoplasia of the hematopoietic stem/progenitor cells characterized by disturbed hematopoiesis in megakaryocytes (MK) development and platelet (PLT) activation. Mutations in 3 genes (JAK2, CALR, MPL) and chronic inflammation are the main pathogenic drivers of MF. Ruxolitinib (RUX), a JAK1/2 inhibitor, suppresses both clonal myeloproliferation and release of proinflammatory cytokines, reducing splenomegaly and constitutional symptoms in around 50% of patients (pts). We hypothesized that MPs, as mediators of inflammation, could be overexpressed in MF and possibly predict responses to RUX.

Aims: This study aims to: 1) enumerate circulating MK and PLT-derived MPs of MF pts; 2) evaluate the effect of RUX on MPs production by PLT and MK; 3) investigate whether circulating MK and PLT- MPs may be a biomarker of response to RUX.

Methods: EDTA-anticoagulated peripheral blood from healthy donors (HD, n=10) and JAK2V617F positive MF pts (n=12) at intermediate-2 high IPSS risk was collected at baseline and 3 and 6 months after RUX therapy and immediately centrifuged. MPs (normal MPVs in megakaryocytes (MK) development and platelet (PLT) activation. Mutations in 3 genes (JAK2, CALR, MPL) and chronic inflammation are the main pathogenic drivers of MF. Ruxolitinib (RUX), a JAK1/2 inhibitor, suppresses both clonal myeloproliferation and release of proinflammatory cytokines, reducing splenomegaly and constitutional symptoms in around 50% of patients (pts). We hypothesized that MPs, as mediators of inflammation, could be overexpressed in MF and possibly predict responses to RUX.

Aims: This study aims to: 1) enumerate circulating MK and PLT-derived MPs of MF pts; 2) evaluate the effect of RUX on MPs production by PLT and MK; 3) investigate whether circulating MK and PLT- MPs may be a biomarker of response to RUX.

Methods: EDTA-anticoagulated peripheral blood from healthy donors (HD, n=10) and JAK2V617F positive MF pts (n=12) at intermediate-2 high IPSS risk was collected at baseline and 3 and 6 months after RUX therapy and immediately centrifuged. MPs (normal MPVs) were analyzed in PLT poor plasma samples by flow cytometry (CytoFLEX, Flow Cytometer-Becman 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 pts (17±6 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±5; p<0.05) and HD (64±7 vs 11±1; p<0.001). Of note, NR pts had significantly lower PLT number as compared with R (220±29 vs 64±7; p<0.001) and NR pts had significantly lower PLT number as compared with R (64±7 vs 17±6; p<0.001). Of note, NR pts had significantly lower PLT number as compared with R (220±29 vs 11±1; p<0.001). The difference was observed at baseline and according to 2013-IWG-MRT criteria. 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 pts (17±6 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±5; p<0.05) and HD (64±7 vs 11±1; p<0.001). Of note, NR pts had significantly lower PLT number as compared with R (220±29 vs 64±7; p<0.001) and NR pts had significantly lower PLT number as compared with R (64±7 vs 17±6; p<0.001). Of note, NR pts had significantly lower PLT number as compared with R (220±29 vs 11±1; p<0.001). The difference was observed at baseline and according to 2013-IWG-MRT criteria. 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 pts (17±6 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±5; p<0.05) and HD (64±7 vs 11±1; p<0.001). Of note, NR pts had significantly lower PLT number as compared with R (220±29 vs 64±7; p<0.001) and NR pts had significantly lower PLT number as compared with R (64±7 vs 17±6; p<0.001). Of note, NR pts had significantly lower PLT number as compared with R (220±29 vs 11±1; p<0.001). The difference was observed at baseline and according to 2013-IWG-MRT criteria.
Results: 110 patients diagnosed with PMF according to WHO criteria between years 2013 and 2014 were included into this study. SNP-A analysis identified 77 chromosomal abnormalities in 61 patients (55.4%). These comprised the loss of heterozygosity (LOH) (59.7%), hemizygous deletions (23.4%) and copy number gains (16.9%). The most common aberrations in affected patients were: 5p LOH (55.7%), 20q deletion (11.5%), 1q duplication (4.9%), 19p deletion (3.2%), 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, ETV6, SF3B1, IDH1, IDH2. Recurrent specific mutations were identified in 10 genes. Sixty-two patients (57.4%) had more than one mutation. Recurrent specific mutations were primarily type 1 (54%) and type 2 (30%).

Summary/Conclusions: A number of associations between gene mutations and chromosomal aberrations was detected in PMF. Co-presence of 9p LOH with JAK2V617F and CALR mutations with 19p deletion indicate that further deregulation of these key signaling pathways may take place disrupting the second allele of the affected genes by different biological mechanism – LOH or deletion.

E1312
FREQUENCY OF CONCURRENT BCR-ABL1, JAK2, CALR AND MPL MUTATIONS IN A COHORT OF 5,545 CASES WITH SUSPECTED MPN BY A DEEP SEQUENCING APPROACH
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Background: Chronic myeloid leukemia (CML) is a myeloproliferative neo-plastic condition characterized by BCR-ABL1 in about 90% of BCR-ABL1-negative MPN a mutation in CALR, JAK2 or MPL can be detected. These genetic alterations are thought to be nearly mutually exclusive, however, an accurate frequency is still missing.

Aims: To determine the incidence of genetic markers occurring in parallel in a large cohort of patients with suspected MPN and characterize double mutated cases.

Methods: From July 2016 till January 2017 5545 samples were sent to our laboratory with suspected MPN. The male:female ratio was 1:1, and the median age was 60 years (range: 18-95 years). Median white blood cell count was 9x10^9/L, hemoglobin level (Hb) was 15g/dl, and platelet count 329x10^9/L. All of these cases were analyzed by an amplicon deep sequencing approach for mutations in JAK2 (exon12, exon14), CALR (exon9) and MPL (exon10) with a sensitivity of 1%. 3070 patients were additionally screened for BCR-ABL1 fusion by a multiplex PCR approach. Samples that were double mutated for JAK2, CALR and MPL were analyzed by amplicon deep sequencing for additional mutations in 13 myeloid genes.

Results: In total 1775/5545 (32%) of suspected MPN patients showed JAK2, CALR and/or MPL mutations. 1438 (26%) were JAK2(+), 267 (5%) were CALR, and 89 (1%) MPL mutated. Of note, the analysis of a subgroup (n=3070) for BCR-ABL1 fusion identified 123 (4%) as CML cases. The JAK2 mutated cases presented mainly with Va117Phe (99%) and rarely with JAK2 exon12 mutations (1%). CALR mutations were primarily type 1 (54%) and type 2 (30%). MPL mutations were located at amino acid Trp515 in 96% of cases. Double mutated cases were present in 19/1775 (1%) cases: JAK2/MPL (63%), JAK2/CALR (32%) and CALR/MPL (6%). In nearly all CALR mutated cases (67%) the affected gene 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 (0.006%). 18/19 patients were analyzed by the deep sequencing approach. All cases that already received treatment for CML 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 (63%) double mutated patients 46/48 (10%) mutations were detected in 8 different genes. SS3FS2 and TET2 were the two most frequently mutated genes (5, 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 (mean: 649 ± 210 x10^9/L, p<0.001). Overall, the mutated cases showed a statistically significant 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 mutations to BCR-ABL1 fusion and/or MPL mutation in an unselected cohort with suspected MPN. The frequency of double mutated JAK2, CALR and MPL cases is 1%. In CML cases BCR-ABL1 fusion and JAK2 or CALR mutation were detected in 2% of the patients. The impact of these parallel genetic events on the clinical course of the disease has to be evaluated in the future.

E1313
A COMPREHENSIVE ASSESSMENT OF MOLECULAR AND CYTOGENETIC MARKERS OF PROGNOSIS IN PATIENTS WITH PRIMARY MYELOFIBROSIS
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Background: According to resent reports the data of molecular and cytogenetic analysis (type or absence of driver mutation (DM), mutations in ASXL1, EZH2, IDH1/2 genes, karyotype) is a promising tool for prediction of survival in primary myelofibrosis (PMF). Multiple combinations of genomic aberrations lead to clinical course and survival heterogeneity. The aforementioned factors need to be considered together to evaluate their mutual influence.

Aims: The aim of the study was to evaluate a prognostic impact of DM, mutational status of epigenetic regulation (ER) genes, karyotype and their combinations for overall survival (OS) in PMF patients.

Methods: We have examined 110 patients (pts) with PMF (34.5% males). Median (Me) age was 59 years (16–82). For all pts the detection of JAK2V617F was done. JAK2(+) samples were tested for MPL 515 codon mutations and exon 9 mutations of CALR (direct sequencing). All pts except 4 underwent the analysis of mutations in ASXL1, EZH2, IDH1/2 genes with high resolution melting method followed by sequencing of probably mutated samples. Karyotype research was done for 48 (43.6%) pts.

Results: DM were detected in 81.8% pts: JAK2(+) - 50%, CALR(+) - 25.5%, MPL(+) - 6.4% cases. No DM were found in 18.2% pts considered triple-negative (TN). Mutations in ER genes were detected in 20.8% pts. High risk (HR) chromosome aberrations (CH) unfavorable CA4M2, del(6)(q15), add(6)(p25), del(X)(q22), t(X;7)(p21;q11) were found in 27.1% pts. Univariate analysis identified HR karyotype (hazard ratio (HR) 8.2, p<0.001), the absence of DM (HR 8.1, p<0.001) and nonsense and frameshift (hereinafter mut) (HR 2.9, p=0.018) but not missense mutations of ASXL1 (HR=0.378) as being prognostically detrimental for survival. CALR mutations had a favorable impact on survival with borderline significance (HR 0.3, p=0.052). A multivariate analysis included TN, CALR, ASXL1 status and karyotype as covariates revealed an inter-independent prognostic value of HR karyotype (HR=7.4, p<0.001) and ASXL1 mut (HR=2.8, p=0.023). In Cox regression model considering the same covariates except karyotype TN status (HR=2.4, p=0.050) and ASXL1 mut (HR=3.3, p=0.012) but not CALR mutations (HR=0.3, p=0.075) were significant for OS. CALR mutations became significant (HR=0.3, p=0.075) when only ASXL1 mut were included as covariate (HR=3.9, p=0.004). When comparing groups divided by CALR(+)ASXL1 status the shortest OS was noted (p<0.001). The median overall survival of groups was 3.7 years (p=0.011) in CALR(+)ASXL1 wide type (wt) pts seem to have better OS than CALR(+)ASXL1wt (median not reached (with follow up period of 10.1 years) and 3.5 years, respectively, p=0.124). Median OS estimated in pts due to presence/absence of DM and ASXL1 status.
was 0.9 years in TNA SLX1wt, 3.6 years in TNA SLX1wt, 13.8 years in DM(+) ASXL1wt and was not reached in DM(+)/ASXL1wt (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=0.786). The better OS was observed in ASXL1wt pts with low risk (LR) karyotype (Me 6.4 years, p=0.0005). There were no differences in OS of ASXL1wt HR, ASXL1mut LR and ASXL1mut HFs pts (1.4 vs 1.6 vs 1.2 years, p=0.493).

**Summary/Conclusions:** The differences in OS were more statistically relevant in groups divided by TN/ASXL1 and karyotype/ASXL1 status. The presence of ASXL1mut significantly worsens OS in the TN group. OS in pts with any of the findings: HR karyotype or ASXL1 mut – was significantly shorter than in cytogenetically favorable ASXL1wt counterparts.

**E1314**

**JAK2 HAPLOTYPE 46/1 (GGCC) HAS NO EFFECT ON THE PRIMARY RISK OF JAK2 V617F MUTATION, BUT IT STRONGLY POTENTIATES THE PROGRESSION OF GROWN ALLELE BURDEN IN MYELOPROLIFERATIVE NEOPLASMS**

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**Background:** Several research groups have determined that the JAK2 46/1 (GGCC) haplotype in multiple ethnic groups is strongly associated with a predisposition to acquiring JAK2 V617F-positive MPNs. The role of the JAK2 46/1 haplotype in the natural evolution of the mutant JAK2V617F allele burden in PV but not ET or PMF has been shown [Alvarez-Larrán A e.a. Leukemia 2012, 36(3):324-326]. However, the data on the impact of the haplotype on the JAK2 V617F allele burden do not always agree. Using a highly sensitive test allowed to reveal a high prevalence JAK2 V617F among persons without symptoms of hematological disorders [Krichevsky S e.a. Blood Cells, Molecules and Diseases, doi: 10.1016/j.bcmd.2017.01.001]. Influence of haplotype 46/1 for such cases is not known. There are two competing hypotheses of "hypermutability" and "fertile ground" explaining the causes of the higher frequency of mutations of JAK2V617F in haplotype 46/1 carriers. The "hypermutability" hypothesis refers to an increased risk of primary mutation in carriers of haplotype 46/1. In this case, the increasing frequency of the haplotype in patients with low allele burden (<5%) must also be observed, including those individuals without evidence of hematological disorders.

**Aims:** Studying the relations of haplotype 46/1 and JAK2 V617F allele burden

**Methods:** The diagnosis of chronic myeloproliferative neoplasms was based on the WHO (2008) criteria. The cohort included patients with JAK2 V617F mutation: 100 patients with PV, 51 with ET, 14 with MF, 41 patients with unclassified Chronic Myeloid Neoplasms (CMN), 52 patients with BCR-ABL1-positive Chronic Myeloid Leukemia, 29 with Acute Myeloid Leukemia (AML) and 3 patients with undifferentiated AML.

**Results:** The JAK2 46/1 haplotype (GG and GC) was present in 170 patients (80.6%) with MPN, in 25 (52%) patients with suspected MPN, in 23 (49%) asymptomatic JAK2 V617F+ pts 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. JAK2 V617F allele burden was significantly higher in patients with PV than in patients with ET (p=0.001), but no differences were observed with from patients with the PMF. 46/1 haplotype was closely associated with MPN patients if the allele burden exceeds 5% (Fig. 1) regardless of the phenotype or the treatment. In this case with an increase in JAK2V617F allele burden the JAK2 46/1 haplotype frequency significantly increased. However, there was no significant difference in the JAK2 46/1 haplotype frequencies between patients with allele burden less than 5% and the control group.

**Summary/Conclusions:** No significant differences of the carrier haplotype 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 allele load is more pronounced in carriers of haplotype 46/1 that supports the "fertile ground" hypothesis. We hypothesize that DNA mutation JAK2V617F repair is downregulated in carriers of haplotype 46/1 that supports the "fertile ground" hypothesis.

**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. Patients’ prognosis is widely variable and the mortality rate can vary from 24% to 40% at 5 years. 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 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 assay 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, Madison, WI, USA) and were evaluated for JAK2V617F mutation both by conventional allele specific PCR (ASO-PCR) and by a validated ddPCR mutation detection assay (Bio-rad, USA). Results were expressed as percentage of JAK2V617F mutated alleles on total evaluated alleles.

**Results:** The JAK2V617F ddPCR mutation assay was able to detect low muta- tion load (up to 0.006%), confirming to be much more sensitive than ASCT (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 0.006%) could be detected by ddPCR in the 2 years after ASCT. With a longer follow-up, a full molecular remission was achieved as demonstrated by ddPCR. In 2 other patients, we observed a very early achievement of full donor chimerism and JAK2V617F molecular negativity (within 90 days post HSCT), also when evaluated by ddPCR. These patients entered a complete hematologic remission of the disease which still persists (after 1 and 5 years after transplantation, respectively). Interestingly, in one patient whose post-transplant hematopoiesis proved full donor and negative for JAK2V617F mutation for 2 years, a weak positive signal revealed by ddPCR (0.075%) became apparent after a subsequent ASCT. 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 patients who underwent ASCT. However, the use of this highly sensitive PCR should be considered with caution in the clinical management of transplanted patients to avoid inappropriately use of donor leukocyte infusion (DLI) and tampering of immunosuppression. A large
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 VERA
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Background: S100A8 proteins have been shown to regulate cell proliferation, excessively augmented in myeloproliferative neoplasms (MPN). S100A8/9 is produced by cells of myeloid origin as mediator of inflammation, while AKT and MAPK pathways mediate cell proliferation.

Aims: This study analyzed activation of AKT and MAPK pathways by S100A8/9 proteins in healthy controls and MPNs: polycythemia vera (PV), essential thrombocytopenia (ET), primary myelofibrosis (PMF), according to JAK2V617F and CALR mutation status.

Methods: S100A8/9 factor is examined in granulocytes of MPN using immunoblotting, while its influence on cell cycle of granulocytes is determined by flow cytometry. Mutations of JAK2V617F and CALR exon 9 are analyzed by DNA sequencing. Besides JAK2V617F+/PV patients, we formed three groups of patients: JAK2V617F+, JAK2V617F-/CALR+, and JAK2V617F-/CALR- for ET and PMF.

Results: S100A8/9 proteolites 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 medi- ated MAPK activation has been significantly augmented by TLR4 and RAGE inhibition in PV patients. S100A8/9 stimulated granulocyte cycle arrest in G2M phase has been stopped by JAK1/2 inhibition.

Summary/Conclusions: S100A8/9 protein levels demonstrated stable elevation in MPN patients. Inhibition of AKT 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 THROMBOCYTEMIA BY HIGH-DEPTH NEXT GENERATION SEQUENCING (NGS)
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Background: Essential thrombocytemia is one of the three classical philadelphia negative myeloproliferative neoplasms. It is frequently difficult to diagnose and some molecular markers are used as diagnostic criteria according to WHO classification. Despite this, a significant proportion of patients do not present a clonality marker.

Aims: To identify the mutational profile of ET negative for V617F and CALR mutations and to correlate it with clinical diagnosis.

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 at diagnosis, 3 patients suffered thrombotic event after diagnosis, 1 patient suffered transformation to AML. Median Hb, WBC and platelets at diagnosis were respectively 14.75g/dl, 8.5 x1012/L and 720 x109/L. We performed targeted gene sequencing by NGS (Ion Torrent Proton System–Life Technologies) using a panel of 33 genes implicated in leukemia prognosis. X2 and t-student tests were used to find association between mutations and clinical data.

Results: On average, 97.94% of the target sequence showed a mean depth coverage around 2500. We discovered 17 non-synonymous mutations which 16 were somatic single nucleotide variants (SNVs) and 1 a nucleotide deletion in coding regions. No mutations were detected in 9 samples (40.9%), 10 samples suffered a somatic mutation (45.5%), and the other 3 samples presented 1 or more mutations (16.3%). TET2 was the most frequently mutated gene (18.2% of patients, mean allele frequency of 24.45%), followed by JAK2 (13.6% V617F at a low mean allele frequency of 5.8%), MPL (9.1%, one W515L, one with two mutations W515R and S505C, mean allele frequency of 21.95%), SF3B1 (4.5% DNMT3A (4.5%), JAK1 (4.5%), MPL (4.5%) and KMT2A (4.5%). The samples with more than one mutation: one presented a CBL and two TET2 mutations, one two mutations in MPL and the other one mutation in TET2 and other in JAK2. No correlation was found between mutational profile and clinical data.

Summary/Conclusions: In ET, around 60% of patients present the JAK2V617F mutation, 15-30% show CALR mutations and around 5% present MPL mutations. In spite of this, there is still a significant percentage of ET patients without a molecular marker. Our study shows that the use of a NGS panel allows identifying markers of clonality as for example TET2. NGS also makes affordable to interrogate whole genes classically associated to ET, to detect mutations that were not found by traditional approaches. Finally, we can conclude, as previously described, that ET is an entity with a low mutational burden in comparison with other MPNS as primary myelofibrosis.

E1318

TGF GAMMA CLONALITY ASSESSED BY NGS DOES NOT HELP TO DISTINGUISH EGPA FROM HES
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Background: Hypersinophilic associated syndromes are a heterogeneous group of diseases characterized by sustained and elevated blood eosinophilia with evidence of eosinophil-induced organ damage. Classically, Eosinophilic Granulomatosis with Polyangiitis (EGPA) and Hypereosinophilic Syndrome (HES) present several overlapping clinical and laboratory features, making it challenging to correctly insert patients in restricted and well-defined categories with specific and more effective therapeutic approaches in daily practice. Therefore, great efforts are ongoing searching for novel biomarkers able to differentiate these two disorders.

Aims: To detect T cell receptor gamma (TCRG) clonal rearrangements in EGPA and HES, comparing the frequency of distribution of the V and J region segments in 21 patients afferent to the hematology, rheumatology or pulmonology divisions.

Methods: Consecutive patients with a diagnosis of EGPA and HES were enrolled into the study. Inclusion criteria were: documentation of a persistent peripheral eosinophilia and at least two organ involvement. Clinical and laboratory data of the patients were collected. Sequence-based determination of the frequency distribution of TCRG Gene Rearrangements was performed using next-generation sequencing with the illumina MiSeq (LymphoTrack TCR analysis). The following TCRG gene rearrangements were observed: Vg10JgP1 (5 cases) and Vg4Jg1/2 (4 cases) were observed in both EGPA and HES. No association was observed between TCRG clonal rearrangements and ANCA status in EGPA patients. Number of following recurrent TCRG gene rearrangements were observed: Vg10JgP1 (5 cases) and Vg4Jg1/2 (4 cases) were observed in both EGPA and HES, whereas Vg9Jg1 (2 cases) and Vg10Jg1/2 (2 cases) were observed only in patients with HES. The presence of TCRG rearrangement was not different according to the symptoms (asthma, vasculitis, skin, heart, gut, lung involvement, splenomegaly). IL2, IL5, IL4, eosinophilic cytokine (ECP), absolute eosinophils were measured: IL5 and ECP were higher in the EGPA patients. This study confirms our previous results showing the TCR delta rearrangement (assessed by qualitative PCR) in 40% of the EGPA patients.
Background: We already demonstrated augmented proinflammatory IL-6 and angiogenic vascular endothelial growth factor (VEGF), hypoxia inducible factor-1α (HIF-1α) and endothelial nitric oxide synthase (eNOS) levels in myeloproliferative neoplasms (MPN).

Aims: To observe IL-6 activated signaling pathways during stimulation of angiogenic factors and their JAK-STAT dependence in MPN.

Methods: We analyzed phosphorylation of JAK/STAT3, PI3K/AKT and MAPK signaling by immunoblotting in HEL 92.1.7 cells (with JAK2V617F mutation) and granulocytes of MPN. The granulocyte cycle phases have been studied by flow cytometry.

Results: We demonstrated IL-6 stimulated angiogenic factors in HEL cells and HEL-derived macrophages, blocked by JAK-STAT inhibition for eNOS and HIF-1α. IL-6 stimulated JAK-STAT3 and angiogenesis related PI3-AKT signaling pathways in HEL cells, the later one prevented by JAK1/2 inhibition. Opposite to primary myelofibrosis (PMF), IL-6 activation of JAK-STAT3 and PI3-AKT pathways has been prevented and enhanced by JAK1/2 inhibition, respectively in granulocytes of polycythemia vera (PV). Moreover, IL-6 inhibition of JAK-STAT3 and PI3-AKT pathways in essential thrombocythemia (ET) has been prevented by JAK2 inhibitor in JAK2V617F positive ET granulocytes. JAK1/2 inhibitor Ruxolitinib upregulated IL-6 activators of MAPK pathway in PMN, in contrast to specific JAK2 inhibitor Hexabromocyclohexane. IL-6 mediated reduction in the percentage of HEL cells in G2M phase was inversed by Ruxolitinib that potentiated apoptosis and reduced the cell percentage in G0G1 phase both in HEL cells and granulocytes of PMF. It has been detected the cell cycle arrest of MPN granulocytes in S phase (DNA replication) after treatment with IL6, completely diminished by JAK1/2 inhibition.

Summary/Conclusions: Therefore, we concomitantly revealed that inflammation stimulated angiogenic factors and signaling pathways involved in cell proliferation, apoptosis and angiogenesis are regulated by JAK-STAT inhibition.

Myeloproliferative neoplasms - Clinical

E1320

PERCEPTION OF SYMPTOM BURDEN AND TREATMENT GOALS BETWEEN PHYSICIANS AND PATIENTS WITH MPNs: AN ANALYSIS FROM THE INTERNATIONAL MPN LANDMARK SURVEY

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Background: The global MPN LANDMARK survey evaluated the patient (pt) and physician-reported impact of myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), among pts from 6 countries. We present an analysis comparing physician and pt perceptions of the impact of these MPNs.

Aims: To investigate differences between pt and physician perceptions of symptom burden, treatment goals, and disease management

Methods: This was a cross-sectional survey of pts with MPNs and physicians treating pts with MPNs. Respondents completed an online survey measuring their perception of the impact of MPNs on symptom burden, treatment management, and treatment goals. Pts and physicians were recruited independently.

Results: Pts (n=699) from Australia (n=10), Canada (n=64), Germany (n=149), Italy (n=106), Japan (n=84), and the UK (n=286) completed the survey (MF, n=223; PV, n=174; ET, n=302). Most pts had been diagnosed within ≤2 years of experiencing symptoms (73%); 56% were women. Physicians (n=219) were from the same countries; most were hematologists (54%) or hemato-oncologists (27%). Overall, 54% of pts reported having a prognostic score; however, 71% of physicians reported using a prognostic risk classification. Physicians assessed symptoms by proactively asking pts how they were feeling (43%) or asking about specific symptoms (37%); 11% waited for pts to mention symptoms. Importantly, only 26% of physicians used a validated symptom assessment form; 44% used their own rating method. Pts and physicians both agreed that pts with MPNs have a high symptom burden and that MF had a higher degree of burden on daily living. Interestingly, a higher proportion of physicians...
than pts felt that MPN symptoms have an impact on pt quality of life (92% vs 76%) and that pts had a substantial emotional burden associated with their disease. For instance, 34%, 29%, and 26% of pts with MF, PV, or ET reported feeling anxious or worried compared with 70%, 46%, and 36% of physicians reporting that their pts experience substantial anxiety or worry. Some pts did not recognize that their symptoms could be MPN related; for example, ≈ one-fifth of pts did not think that their night sweats could result from their MPN (16% MF, 21% PV, 25% ET). Consistent with this, 60% of physicians indicated that pts could identify only few or some of their symptoms as MPN related. Pts and physicians were both concerned about reducing symptoms (pts: 70% MF; 61% PV, 53% ET; physicians: 80% MF, 55% PV, 60% ET); however, pts were also concerned about delaying MPN progression (58% MF, 57% PV, 66% ET; physicians: 43% MF, 28% PV, 37% ET; Figure 1). Compared with pts, physicians indicated a greater focus on prevention of vascular/thrombotic events in PV (66% vs 48%) and ET (80% vs 60%). Overall, only 27% of physicians felt they completely agreed with their pts on treatment goals; 66% felt they “somewhat” agreed. However, most pts (87%) were satisfied with their physician’s disease management/communication.

Summary/Conclusions: This study revealed a potential disconnect between physician and pt perceptions relating to communication and disease management, and an apparent lack of standardization in symptom assessment. Of note, some pts did not recognize that their symptoms could be MPN related and had different treatment goals than their physicians, indicating a need for improved pt education and pt-physician communication and a treatment plan that includes standardized monitoring of symptoms and agreement on treatment goals.

Figure 1.
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), rouxolitinib 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 hallmark by hyperactive inflammatory pathways and constitutional symptoms.

Aims: We aim to closely evaluate the significance of SA in MF patients as it pertains to clinical presentation, laboratory correlations, disease genomics, comorbidities and outcomes.

Methods: We retrospectively reviewed an institutional database of 376 MF patients who presented to Moffitt Cancer Center between 1/1/1998 and 12/31/2012 and had available SA levels within 30 days of presentation. Laboratory values and prognostic scores were determined at time of first presentation. Overall survival (OS) was measured from time of first presentation until date of death or censored at time of last follow-up. Progression free survival (PFS) was defined as time from first presentation to development of acute myeloid leukemia (AML).

Figure 1.

Results: Our cohort of MF patients had median age of 67 and 69 at diagnosis and presentation, respectively. Most patients had primary MF (73%) with 11% and 16% having post-PV PMF 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 packs-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=89); cohort III=SA 4.1-4.5 g/dL (n=182); and cohort IV=4.6 g/dL (n=84). OS increased with increasing SA; with median OS in cohorts 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 patients diagnosed 2012-2013 versus 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.

E1324

CLINICAL UTILITY OF NEXT-GENERATION SEQUENCING IN THE MANAGEMENT OF MYELOPROLIFERATIVE NEOPLASMS

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Background: Although Next Generation Sequencing (NGS) has helped characterize the complex genomic landscape of myeloid malignancies, its clinical utility remains not well defined. Funding for NGS testing by healthcare systems or third party payers is variable due to the lack of data on its utility in a routine care setting. At our centre, targeted sequencing (TAR-seq) is offered to all new patients referred for myeloid malignancies as part of the Advanced Genomics in Leukemia (AGILE) program.

Aims: In this study, we evaluate the impact of TAR-seq on the management of patients with a diagnosis of MPN or post-MPN acute myeloid leukemia (MPN/AML).

Methods: All consenting patients referred to the MPN program at the Princess Margaret Cancer Centre between February 15 and December 31, 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 covers) implicated in myeloid malignancies. Reporting was confined 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), 13 with other MPN and 12 with unspecified MPN. Of the remaining 12 with 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 clonal hematopoiesis through identifying other mutations. In 61 transplant-eligible patients with MF, 32 (52%) were considered to carry a high molecular risk profile based on scoring at various centers in AML, AML, EZH2, IDH1/2, SRSF2 or TP53; or a total of three or more mutations. Of these, 11 patients (34%) were considered for early transplant, three with Intermediate-1 and eight with Intermediate-2 risk, who were responding well to JAK 1/2

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implications. 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.

E1325
IMPACT OF COMORBIDITIES AND BODY MASS INDEX ON SURVIVAL IN PATIENTS WITH MYELOFIBROSIS TREATED WITH RUXOLITINIB
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Background: Charlson Comorbidity Index (CCI) and body mass index (BMI) are significantly associated with outcome in patients (pts) who receive continue treatment with tyrosine kinase inhibitors. Ruxolitinib (RUX) is the first JAK1/2 inhibitor that may induce spleen/symptom responses and improve quality of life in pts with myelofibrosis (MF). No data are yet available on the impact of comorbidities and BMI on pts treated with RUX.

Aims: To evaluate the impact of CCI and BMI on overall survival (OS) in a cohort of RUX-treated MF pts.

Methods: A multicenter observational study on WHO-defined MF treated with RUX according to standard clinical practice was conducted in 20 Italian Hematology Centers. Response to RUX was evaluated according to 2013 IWG-MRT criteria. OS was calculated from the date of RUX start to the time of death or last follow-up. Baseline parameters evaluated for correlation with OS were: blood cell count, spleen ≥10cm, marrow fibrosis grading, time from MF diagnosis to RUX start, transfusion dependency, mutation status, Total Symptom Score (TSS), CCI and BMI.

Results: Between June 2011 and Apr 2016, 343 pts with PMF (51.9%), or post-ET (20.1%) / post-PV (28.0%) were treated with RUX in participating Centers. At RUX start, median age was 67.6 years (range 35.6-89.0) with a male prevalence (57.1%); International Prognostic Score System (IPSS) was interme- diate (intm-1) (16.0%), intm-2 (47.5%), high (38.4%). Transfusion dependency (HR: 1.74; p=0.039) and IPSS (intm-2; HR: 3.19; p=0.057; high risk: HR: 6.89; p<0.001) were significantly correlated with OS (Fig. 1A). Also, a higher CCI did not correlate with lower spleen response at 6 months (44% vs 34% of pts with CCI<3, p=0.11). The impact of higher CCI on survival was only mildly affected by the achievement of a spleen response at 6 months (Fig. 1B).

Figure 1.

Summary/Conclusions: Together with transfusion requirement, CCI and BMI may influence survival in RUX-treated MF pts. Taking into account these additional parameters may allow to better define survival probability beyond IPSS risk assessment. Unfavorable CCI and BMI did not hamper responses to RUX; also, the achievement of a spleen response counterbalanced the negative prognostic effects of a lower BMI.

E1326
ANALYSES OF 845 PATIENTS WITH PMF, PET-MF AND PPV-MF TREATED IN 35 GERMAN HEMATOLOGY CENTERS – A RETROSPECTIVE FIELD STUDY
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Background: Primary myelofibrosis (PMF) as well as secondary post essential 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, treatments, comorbidities and outcome, was sent to all centers in 2017. The questionnaire included 178 questions which were divided into 5 categories: patient characteristics, disease characteristics, procedure details, therapy details and outcome.

Results: 35 hematology centers participated in the study. A total of 845 patients were included, 477 with PMF (56.5%), 208 with post-ET-MF and 160 with post-PV-MF. The median age at diagnosis was 68 years (range 18-93). The median time of diagnosis to questionnaire was 7 years (range 0-34). The primary malignant disease was myelofibrosis in 717 patients (85.0%), myelofibrosis associated with another malignant disease in 53 patients (6.3%), and other malignant diseases in 75 patients (8.8%). The median number of comorbidities was 1 (range 0-3). The median number of transfusions was 3 (range 0-28). The median number of all hematopoietic SCT was 1 (range 0-10).

Summary/Conclusions: The questionnaire shows that the primary malignant disease was myelofibrosis in the majority of patients (56.5%), followed by post-ET-MF (20.8%) and post-PV-MF (19.7%). The median age at diagnosis was 68 years (range 18-93). The median time of diagnosis to questionnaire was 7 years (range 0-34). The median number of comorbidities was 1 (range 0-3). The median number of transfusions was 3 (range 0-28). The median number of all hematopoietic SCT was 1 (range 0-10).
was 03/2013-2015. 845 pts were included i.e. a median of 20 pts (range 6-90 pts) per center

**Results:** Gender was equally distributed (50%/50%). Pts ages at initial diagnosis as were as follows:<50 years (31%), 50-69 years (31%), 70-79 years (31%), and ≥80 years (40%). Current age was >65y in 70% of all pts. PMF represented the largest MF cohort (77%), followed by Ph-MPN (10%), PV-MF (7%) and unspecified (6%). Median survival was 5.3 years (range 1-month-24 years). Significant differences were observed in the distribution of the following subgroups: less pts younger than 50 and more pts over 70 years old in the Ph-MPN group, more pts younger than 70 years old in the PV-MF group and in the unspecified group, and more pts younger than 65 years old in the PMF group. The majority of pts had myelofibrosis (61%).

**Conclusion:** Our study showed that the majority of pts in this Spanish cohort had MF with myelofibrosis. The median survival was 5.3 years and the majority of pts at diagnosis had myelofibrosis. The current age distribution was different from other published studies where younger ages were more common in the Ph-MPN group and older ages in the PMF group. Further studies are needed to determine the impact of age on survival in MF.

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**JAK2V617F group showed higher hemoglobin values (mean 13.8g/dL; p=0.014) and lower platelet counts (mean 730x10^9/L; p=0.014) than CALR group, for L-selectin levels in all three therapy groups and for P-selectin levels in CALR type-1like group. When comparing CALR type-1like vs CALR type-2like the differences were marginally significant (p=0.06). In a multivariate analysis with the IPSET variables and CALR subtype, in our series the previous history of thrombosis (p <0.001) and the JAK2V617F status (p=0.026) were significantly associated with increased risk of thrombosis, but no the advanced age neither the presence of cardiovascular risk factors. However, presence of CALR type-2 like mutation, with respect to the JAK2V617F mutation, was a protective factor of thrombosis (p=0.06).**

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**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.

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**Figure 1.**

**Summary/Conclusions:** When comparing CALR type-1 like with CALR type-2 like, the differences were marginally significant (p=0.06). In a multivariate analysis with the IPSET variables and CALR subtype, in our series the previous history of thrombosis (p <0.001) and the JAK2V617F status (p=0.026) were significantly associated with increased risk of thrombosis, but no the advanced age neither the presence of cardiovascular risk factors. However, presence of CALR type-2 like mutation, with respect to the JAK2V617F mutation, was a protective factor of thrombosis (p=0.06). The five year-thrombosis free survival (TFS) study was as follows: 83%, 85% and 97% for groups JAK2V617F, CALR type-1 like and CALR type-2 like (log rank p=0.03/fig. 1).
HEAT SHOCK PROTEIN 27 EXPRESSION IS INCREASED IN PATIENTS WITH PRIMARY AND SECONDARY MYELOFIBROSIS AND MAY BE AFFECTING THEIR SURVIVAL

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Background: Increased heat shock protein 27 (HSP27/HSPB1) expression and phosphorylation were observed in a large number of neoplastic diseases and they have mostly been associated with aggressive disease features and poor prognosis. There are only few reports investigating HSP27 in primary myelofibrosis (PMF), a myeloproliferative neoplasm characterized by high inflammatory state reflecting in debilitating clinical symptoms.

Aims: To analyze HSPB1 mRNA expression in patients with PMF and secondary myelofibrosis (SMF) and to correlate it with clinical and hematological features.

Methods: We analyzed HSPB1 relative expression in bone marrow aspirates of 26 patients with PMF, four patients with SMF and 13 controls using quantitative real time polymerase chain reaction (RT-PCR). Spleen size was assessed by palpation. Association with overall survival was analyzed in 27 PMF and SMF patients evaluated at the time of diagnosis. The 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=0.001); it was significantly higher in patients with PMF and SMF than in control group (P<0.05 for both comparisons), but did not differ between PMF and SMF patients (non significant). Increased expression was associated with increase in the spleen size (P=0.009) and JAK2 V617F mutation (P=0.073).

We did not detect significant associations with other disease specific features. Lower HSPB1 expression was associated with inferior overall survival in both univariate (HR 3.2; P=0.04) and multivariate analysis (HR 6.1; 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).

E1329
NON-DRIVER MUTATIONS IDENTIFIED BY A 190-GENE NEXT GENERATION SEQUENCING PANEL IN PATIENTS WITH PRIMARY MYELOFIBROSIS AND POST-POLYCYTHAEMIC/ESSENTIAL THROMOCYTHEMIA

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Background: It is a consensus that the driver mutation is an independent prognostic factor in PMFs. Moreover, some non-driver mutations are found associated with initiation, progression and prognosis in PMFs. However, a recent study from the AGIMM (AIRC- Gruppo Italiano Malattie Mieloproliferative) group showed that the type of driver mutation did not influence prognosis in post-PV/ET MF. These observations proved that there were indeed some differences in these two types of MF.

Aims: The aim of current study was to describe the non-driver mutation landscape and the molecular differences between the patients with PMF and those with post-PV/ET MF.

Methods: Targeted gene sequencing was carried out at diagnosis. We sequenced 190 genes across 62 patients, resulting in 229 high-confidence mutations. The average gene coverage was 99%. The average read depth was 540×. Also, 92% of targeted regions were covered with >20×. Every mutation identified in this study was then compared against these expected patterns and categorized into “oncogenic,” “possible oncogenic variants,” or “unknown significance”. Using copy number-adjusted VAF, we reconstructed the clonal architecture to establish whether a mutant gene was an ancestral or subclonal mutation. According to the statistically differences in VAF among gene mutations, subjects were classified as two different clonal architecture, namely clone+subclone(s) (P<0.05) or clonal.

Results: In PMFs, 42 (93.3%) patients had at least one non-driver mutation. Within the 17 patients lacking the driver mutations in JAK2V617F/Exon12, MPLW515 and CALR, 2 had mutant genes (SH2B3 and PIAS3) involving in JAK-STAT pathway, 13 had mutations in other genes and 2 had no mutations. In Post-MFs, non-driver mutations were detected in 16 (94.1%) patients. There are no differences in the median number of non-driver mutations in PMFs vs. post-PV/ET MFs (3 vs. 3.18, P=0.885) and PMF patients with vs. without driver mutations (3 vs. 3.18, P=0.668). In PMFs, 12 non-driver genes were mutated in >5% of patients, namely ASXL1 33.3%, U2AF1 22.2%, TET2 15.6%, FAT1 15.6%, SETBP1 13.3%, SRSF2 8.9%, CUX1 8.9%, EP300 8.9%, FAT2 6.7%, NOTCH3 6.7%, EZH2 6.7%, and GATA3 6.7%. In post-PV/ET MFs, ASXL1 (41.2%) was the most frequent mutation, followed by TET2 (29.4%), U2AF1 and SRSF2 mutations were significantly more frequent in PMF than in post-PV/ET MF. Moreover, SETBP1 and FAT1 were mutated in PMF more often and not mutated in post-PV/ET MF. Figure 1 A-C show 3 illustrative patients. Clonal architecture was significantly different between PMFs and post-PV/ET MFs (Figure 1D). About 50% PMF patients were classified as clonal, however, most (87%) post-PV/ET MF patients were clone+subclone(s). In PMFs, driver mutation was an ancestral mutation with other non-driver mutations in 34 (31.1%) subjects as 2015-R02413 in Figure 1A. Moreover, driver mutation even was a subclonal mutation in 9 (16.7%) subjects as 2015-R02406 in Figure 1B.
1B. In post-PV/ET MFs, 11 (64.7%) subjects showed that JAK2 mutation as an only ancestral mutation as G121517R00701 in Figure 1c.

Figure 1.

Summary/Conclusions: In conclusion, we found that the differences in non-driver mutation profile and clonal architecture between PMF and post-PV/ET MF. In addition, by applying a 190-gene panel we demonstrated some variants classified as of “unknown significance”. And larger sample sizes may enable some of these to be reclassified in the future. The precise role of each mutation and their impact on MPN phenotype will require further studies.

E1331

DETERMINING MEANINGFUL CHANGE IN THE MYELOFIBROSIS SYMPTOM ASSESSMENT FORM (MFSAF) v2.0 USING A COMBINATION OF DISTRIBUTION- AND ANCHOR-BASED APPROACHES IN THE COMFORT-I TRIAL

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Background: Symptom response was defined in the COMFORT-I trial as a 50% improvement from baseline at week 24 in the Myelofibrosis Symptom Assessment Form (MFSAF) v2.0 total symptom score (TSS; Mesa [J Clin Onc, 2013]; 0 to 60 scale where 60 represents the worse symptom experience imaginable) with no minimum score requirement at baseline.

Aims: In this analysis of the phase III placebo-controlled COMFORT-I study we used distribution- and anchor-based approaches to investigate whether alternative change scores in the MFSAF v2.0 TSS could be meaningful relative to patient-reported quality of life (QOL).

Methods: One third and one half of the pooled standard deviations (SD) of scores and change scores (raw and percentage change) relative to the patient's change in global health status/QOL (GH/QOL; =worst, 100=best) as measured by the EORTC QLQ-C30 where a decrease of 12.1 or more points was considered as deterioration; an increase of 7.6 or more points was considered as improvement; and all other changes were considered as stable based on change scores established in a multiple myeloma population (Kvam et al., Eur J Hem, 2011). Analysis of covariance (ANCOVA) was used to investigate whether estimated meaningful changes were consistent across the spectrum of observed baseline TSS. This model of TSS changes at week 24 included a continuous term for baseline TSS, a 3-level grouping factor for GH/QOL change (deterioration vs stable vs improvement), and an interaction term between baseline TSS and the GH/QOL grouping factor.

Results: 301 patients randomized to ruxolitinib [N=149] or placebo [N=152] completed TSS at baseline (45% female, median age 68 [range 40-91]). Median baseline TSS was 16.8 (range 0 to 52.7). Pooled SD at baseline and week 24 in TSS was 11.4 and 11.6, respectively, resulting in estimated meaningful changes of 3.8-5.8 points. For change and percentage change from baseline at week 24 in TSS, the pooled SDs were 9.8 and 75%, respectively, resulting in estimated meaningful changes of 3.3-4.9 points or 25%-38%. Among patients with TSS and QLQ-C30 data at baseline and week 24, 51 (23%) patients had deterioration, 61 (27%) were stable, and 110 (50%) had improvement based on QLQ-C30 GH/QOL changes. Mean (95% CI) changes in TSS for the three groups were 0.8 (-2.5 to 4.2), -1.4 (-3.8 to 0.8), and -6.8 (-9.0 to -4.6), and for percent changes 20% (-6% to 46%), 17% (-11% to 44%) and -34% (-45% to -22%), ANCOVA revealed that baseline TSS statistically significantly impacted the change estimates (p=0.02). Figure 1 shows the distribution of estimated meaningful 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 the change estimates (p=0.02). Figure 1 shows the distribution of estimated meaningful 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 the change estimates (p=0.02).

Figure 1.
response duration were 4 and 31 months respectively. 23% of patients lost response after a median time of 16 months. Seventy-five % of patients responded to ruxolitinib in terms of spleen size, of whom 86.4% also achieved an erythropoietin level below 250 UI/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–, Int-2–, and high-risk MF by the International Prognostic Scoring System (IPSS) in the phase 3 COMFORT studies. JUMP is a large, phase 3b, expanded-access trial in countries with no access to RUX outside a clinical trial and includes pts with IPSS Int-1–, Int-2–, and high-risk MF. To further evaluate RUX, we conducted an analysis assessing safety and efficacy of RUX by Dynamic IPSS (DIPSS) prognostic risk.

Aims: To compare the safety and efficacy of RUX in pts with DIPSS low/Int-1– vs Int-2– vs high-risk MF

Methods: Eligible pts had IPSS high- or Int-2–risk MF, or Int-1–risk MF and a palpable spleen (>2 cm). Starting dose was based on baseline platelet (PLT) count (5mg bid [50 to <100×10^9/L], 15mg bid [100-200×10^9/L], or 20mg bid [>200×10^9/L]) and could be titrated during treatment. The primary endpoint was safety and tolerability of RUX. Changes in palpable spleen length and symptom scores were also assessed. DIPSS scores were determined using pt characteristics at baseline.

Results: Based on available pt data, DIPSS status was determined for 1840 of 2233 enrolled pts. JUMP included 893 low/Int-1–, 754 Int-2–, and 193 high-risk pts (primary MF, 57%, 63%, 62%) who started treatment ≥1 y before data cutoff (01 Jan 2016). Pts with higher-risk MF were older (62, 68, and 72 y), had lower Hb (<10 g/dL, 3%, 64%, 100%), and had higher blast counts (<1%, 18%, 44%, 84%). 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 (88%, 57, 59%) or 15mg bid (26%, 32%, 33%). Median exposure was 16, 11, and 9 mo; median average daily dose was 30, 28, and 29mg. At data cutoff, most pts remained on treatment or had completed per protocol (70%, 52%, 25%). Main reasons for treatment discontinuation included adverse events (AEs; 15%, 11%, 6%), disease progression (6%, 11%, 11%), and death (2%, 5%, 11%). The most common hematologic grade 3/4 AEs were anemia (22%, 44%, 55%) and thrombocytopenia (11%, 18%, 25%), but these rarely led to discontinuation. Overall rates of nonhematologic grade 3/4 AEs were <2%, except for pneumonia (4.5%), pyrexia (2.3%), asthenia (2.2%), and dyspnea (2.2%). Infections in <5% of pts were pneumonia infection (7.3%), urinary tract 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 >50% reduction from baseline in spleen length; 19% (68/355), 19% (43/232), and 23% (12/52) had 5%-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 (2.9-75 wk), 5.3 wk (2.6-80 wk), and 8.1 wk (3.1-72.3 wk). From wk 4 to 48, 39%, 43%, 41%, and 48%, and 48% of pts achieved a clinically meaningful response on the FACT-Lym TS; proportions of responders on the FACT-Fatigue were 42%-49%, 46%-49%, and 55%-61%.
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 10mg bid. The primary endpoint was safety. Secondary end-points included changes in spleen length and symptoms.

### Results:
A total of 48 pts (primary MF, 60%) started RUX at 10mg bid ≥1 y before data cutoff (01 Jan 2016). Mean baseline characteristics were: median age, 65.5 y (range, 20-83 y); male, 44%; spleen length, 12.3 cm; time since diagnosis, 56.6 mo; hemoglobin (Hb), 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 same was true for treatment discontinuations due to skin toxicity (16/120 [13%] vs 52/120 [43%] compared to non-HU treatment (52/120 [43%] vs 9/126 [7%]; p=0.0001), and the same was true for treatment discontinuations due to skin toxicity (16/120 [13%] vs 3/126 [2%]; p=0.014).

### Summary/Conclusions:
According to our prospective observation, skin toxicity was clearly associated with HU treatment compared to other cytoreductive drugs. This resulted in a higher rate of HU treatment termination due to skin toxicity. However, median exposure time to HU was longer compared to non-HU treatment (52/120 [43%] vs 9/126 [7%]; p=0.0001), and the same was true for treatment discontinuations due to skin toxicity (16/120 [13%] vs 3/126 [2%]; p=0.014).

### 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 beginning December 2010 and November 2016.

### Results:
In total, 151 MPN pts under cytoreductive therapy were included (ET, n=55; PV, n=55; MF, n=41). Primary MPN diagnosis was made between 1979 and 2012 at a median age of 55 years (range, 22-82). Median duration of the disease until baseline of the study was 6.3 years (0-32.6). Median prospective observation time for the total cohort within the study period was 5.3 years (0.4-6.2). Most frequently used cytoreductive drugs were HU in 120 pts, followed by ruxolitinib in 59, anagrelide in 39, and pegylated Interferon-alpha (IFN-a) in 28 pts. Median cumulative HU exposure was 46 months (1-252), while the median cumulative treatment time for the corresponding drug in the 120 HU pts was 24 months (1-267) [ruxolitinib: 22 months (2-64); anagrelide: 19 months (1-216); IFN-a: 64 months (1-267)]. Of 120 pts exposed to HU, 52 pts (43%) presented with skin abnormalities during the observational period occurring after a total HU treatment time of median 46 months (1-252). Sixteen of 120 pts (13%) discontinued HU due to skin toxicity such as skin ulcers (n=6), phototoxicity / erythrodermia (n=5), actinic keratoses (n=3), dry skin / xerostomia (n=2). Of note, four malignant skin diseases were reported under HU therapy (basal cell carcinoma, n=3; malignant melanoma, n=1). Although pts of the HU cohort were exposed longer to the drug compared to pts of the non-HU group, numbers of skin events in non-HU treated pts were as follows: n=5 under anagrelide (skin ulcers, n=2; allergic reaction, n=2; basal cell carcinoma, n=1), n=4 under IFN-a (local reaction after subcutaneous injection, n=3; actinic keratoses, n=1), and none under ruxolitinib. In 3/126 (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 keratoses’ occurred under combination therapy with HU. Taken together, skin alterations occurred more frequently under HU compared to non-HU treatment (52/120 [43%] vs 9/126 [7%]; p=0.0001), and the same was true for treatment discontinuations due to skin toxicity (16/120 [13%] vs 3/126 [2%]; p=0.014).

### Summary/Conclusions:
According to our prospective observation, skin toxicity was clearly associated with HU treatment compared to other cytoreductive drugs. This resulted in a higher rate of HU treatment termination due to skin toxicity. However, median exposure time to HU was longer compared to non-HU treatment, and controlled clinical trials are necessary to provide more precise data on the occurrence and severity of skin toxicity under HU.

### 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 monocyto-sis during the course of PMF has been associated with a worse outcome, and absolute monocyte counts have been shown to be of prognostic value in other MPNs. Basophilia and eosinophilia are frequent findings in BCR-ABL-
positive MPNs, where they associate with an accelerated phase of disease, and seem to correlate with worse survival in myelodysplastic syndromes. However, the impact of these three findings at diagnosis in PMF remains unclear.

**Aims:** The aim of this work is to evaluate, at diagnosis, the prognostic impact of basophilia, eosinophilia and monocytosis in patients with PMF.

**Methods:** We identified all PMF patients diagnosed and followed-up in our Centre between January 1st 2005 and August 31st 2016, who still fulfilled PMF criteria under the WHO 2016 diagnostic revision, have synchronous bone marrow (BM) and peripheral blood (PB) analyses dating from the time of diagnosis, and have complete charts with no missing data. After the exclusion of reactive causes, monocytosis was defined as an absolute count (AC) ≥20% blasts in bone marrow or peripheral blood) and for whom at least one DNA sample to define the mutational status of the three MPN driver genes (≥ 20% blasts in bone marrow or peripheral blood) and for whom at least one DNA sample to define the mutational status of the three MPN driver genes.

**Results:** We studied 55 evaluable patients (73% male) with a median age at diagnosis of 70.1±11.7 years old. At diagnosis, 20% of patients had monocytosis, with no significant differences according to gender or age. The median overall survival (OS) in PMF patients with monocytosis was 27.3 months, and twice as long (46.4 months) in patients without. In this cohort, a new calculated cut-off of 0.75 G/L was better able to stratify patients according to survival with a specificity of 74.1% (95% CI: 53.7-88.9%); 32.7% of patients had an AC above the cut-off, with a median OS of 27.9 months, compared to 6.4 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 27.3 months, compared to 43.8 months for patients under the cut-off. A total of 30.9% of patients had basophilia at diagnosis, with no differences according to gender or age. The median OS in patients with basophilia was 25.6 months, and 32.5 months in patients without. With a new cut-off of 0.25 G/L of basophils, with a specificity of 88.9% (95% CI: 70.8-97.6%), 20.0% of patients had a basophil AC above the cut-off and a median OS of 19.7 months, compared to 46.4 months for patients under the cut-off. Considering the whole cohort, 61.8% of patients had normal monocyte, eosinophil and basophil ACs; the median OS in these patients was 56.1 months, compared to 28.5 months in patients with an increase in at least one AC. Applying the new cut-offs, OS increased to 79.5 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.

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**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). Clonal 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 MPN in blast phase, according to MPN diagnosis and mutational profile.

**Methods:** We identified in our database all patients affected with ET, PV and PMF who developed acute myeloid leukemia according to 2016 WHO criteria (≥ 20% blasts in bone marrow or peripheral blood) and for whom at least one DNA sample was available to define the mutational status of the three MPN driver genes (JAK2, CALR or MPL). JAK2 and CALR mutational status was determined by Sanger sequencing; MPL (MPL515W) mutation was identified also in AML blasts in all 6 patients for which DNA was available. Outcome was dismal, independently from the driver mutation; the median overall survival (OS) was 9.3 months (range 3.9-15.7) compared to ET (median 36.7 years, range 54.4-86, P 0.318) 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 driver mutation; the median overall survival (OS) was 9.3 months (range 3.9-15.7). OS was not influenced by treatment during blast phase (4.5 months with induction chemotherapy versus 4.6 months with palliation; P 0.885). Of the whole series, only one patient is alive and in complete clinical and molecular remission, after 11 months from allogeneic bone marrow transplantation.

**Figure 1.**

**Summary/Conclusions:** Clinical phenotype and outcome of BP is not influenced neither by the diagnosis in chronic phase nor by the driver mutation; moreover the outcome is poor irrespective of treatment. PMF patients have a shorter time to BP than ET and PV patients; in PMF JAK2 V617F mutation is associated with a shorter time to BP compared to CALR mutation. The only potentially curative treatment is represented by allogeneic stem cell transplantation, but only a few patients can actually undergo this procedure.

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**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%), Calreticulin (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 a marker of aging and genome stability. Hematopoietic cells in several hematological malignancies have been shown to be characterized by shortened TL.

**Aims:** Determine if there is TL shortening in patients with ET when compared to age and gender matched controls and establish the effects of cytoxic and non-cytoxic therapy on TL.

**Methods:** 100 patients were included in the study (27 with CALR, 35 JAK2V617F and two MPL515W mutations. 36 patients were TN). Most patients were female (70% 70/100); median age was 45 years (range 20 - 86 years).
TL was determined in peripheral blood mononuclear cells using a monochrome multiplex quantitative PCR based on the original methods described by Cawthon. All results were corrected for age and gender.

**Results:** Regardless of driver mutation status ET patients had significantly shortened TL compared with age and gender matched controls, p<0.0001. Considering individual mutation status these differences remained significant e.g. JAK2 V617F positive (p=0.007), JAK2V617F vs Placebo (p=0.012) in TN patients. TL appeared more markedly short in the CALR cohort; for the 18 patients, whose TL was below the first centile, 55% (10/18) were CALR positive vs 28% (5/18) JAK2V617F positive vs 17% (3/18) who were TN. Concerning the potential impact of therapies 31/100 patients were treated with hydroxy- and 13/100 with IFN (e.g. five of these had prior exposure to HC). 34/100 were not on cytoreductive therapy. Remaining treatments were ruxolitinib (5), busulphan (4), anagrelide (1) and vonostat (1). Independent of mutation status there was significant TL shortening in untreated patients, p=0.05; however, upon evaluating the impact of cytoreductive therapy on TL we noted that patients who were on either current, or prior HC had significantly shortened telomeres: in untreated patients, p=0.0015 and p=0.0001 respectively. Strikingly, there was no significant difference in TL in IFN patients who had no previous exposure to HC, p=0.2 but those ET patients currently on IFN but with prior HC exposure still had shorter TL.

**Summary/Conclusions:** We document for the first time that ET patients, when compared to age and gender matched healthy controls, have shortened TL. This shortening is more pronounced in CALR and JAK2 V617F positive patients. Concerning therapy whilst present in untreated patients TL shortening was more pronounced in HC treated patients indicating that there may be a therapy effect as has been observed after HC treatment in sickle cell disease. Of note IFN treated patients had more normal TL suggesting that the disease related TL effects may be reversed by this agent.

**Figure 1.**

**Summary/Conclusions:** Nutrition decline remains an unmet need for many MF patients. JAK2 inhibition represents a potential source to improve symptom burden in those who qualify for therapy. Leptin closely correlated with many other nutritional values suggesting this may be a good marker of nutritional status in MPN patients. CRP was inversely correlated with BMI, suggesting the importance of inflammation as a contributor to weight loss. Further study into the unique nutritional needs of myelofibrosis patients is warranted.
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), splenomegaly 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-Whitney 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 cytoeraductive 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.

Figure 1.
Summary/Conclusions: Unfortunately, no improvement, neither as the TFS nor the OS was observed (Fig. 1 and 2): more efforts to better identify the groups at risk and, hopefully, the introduction of new drugs as JAK-2 inhibitors could change the prognosis of ET patients.

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 thrombocytosis 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 pilars (76.2% patients), nail changes (41.3%), actinic keratosis (39.7%), hyperpigmentation of the skin (23.8%), pruritus (23.8%) and non-melanoma skin cancer (22.2%). In figure 1 we detail all the skin alterations that we have found.

Summary/Conclusions: Cutaneous involvement in MPNs is more frequent than expected and it is usually underdiagnosed. Some of these lesions could be prevented with the correct treatment of their pathology and adequate 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 cytoeraductive therapy.

Figure 1.

E1342

HEMOGLOBIN AND WHITE CELL COUNT IN PATIENTS CLINICALLY SUSPECTED TO HAVE ESSENTIAL THROMBOCYTHEMIA MAY HELP IN PREDICTING EARLY PRIMARY MYELOFIBROSIS 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 thrombocytosis (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 ≥13x109/L) or elevated LDH (>200 mU/mL). For an accurate classification, 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 unmet 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.

Methods: A retrospective Personal Practice Assessment Program was conducted at 8 Canadian institutions. Eight hematology/pathology pairs reviewed charts of about 20 consecutive examined patients who presented with thrombocytosis and clinically suspected to have ET. 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 ≥13x109/L) or elevated LDH (>200 mU/mL). For an accurate classification, 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 unmet 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.

Figure 1.
presentation with thrombocytosis. No central pathology review was planned for this stage of the study.

Results: A total of 122 patients (58 males and 66 females; 54% >60 years of age; 65% with LDH >200 mU/mL) with a clinical history indicative of ET were initially assessed. A majority of patients (76%) presented with suspected ET within the last 5 years, likely because it was more difficult for clinicians to identify patients with BM biopsy collected within a year of presentation with thrombocytosis if they presented more than 5 years ago. Out of 122 patients, 48 met the hemoglobin and/or leukocytes 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 met criteria for ET and one third for PMF. While 2 of the remaining patients met criteria for PV, the rest were uncertain whether to represent true ET or early PMF, i.e. represented MPN-U (Figure 1).

Summary/Conclusions: Despite its methodological limitations, this initiative confirms that in real world clinical practice the Carobbio algorithm can be used to identify patients with ET and early PMF and steering clinicians clinically suspected to have ET. It suggests a need for educational initiatives on using diagnostic algorithms to separate ET from PMF. It confirms the importance of hematologist-pathologist collaboration in reaching a final integrated diagnosis based on the WHO classification. These findings warrant investigation in larger prospective studies.

E1343
PK/PD MODELING COMPARING DIVIDED DOSING (200mg TWICE-DAILY [BID]) VS SINGLE DOSEING (400mg ONCE-DAILY [QD]) OF PACRITINIB (PAC) IN PATIENTS WITH MYELOFIBROSIS (MF) ON THE PERSIST-2 PHASE 3 TRIAL

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Background: MF is a life-threatening hematologic malignancy characterized by splenomegaly and debilitating constitutional symptoms. At the present, the JAK2 inhibitor ruxolitinib is the only therapy approved (pts) with hematopoietic stem cell transplantation (HSCT) as a standard for high risk MF. It 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 previously presented (Mas- carenhas et al. Blood 128(22), LBA-5.), showed significantly higher spleen volume reduction (SVR) with PAC (QD and BID pooled) vs QD dosing by 10% (E1343).

Aims: Validate the clinical utility of PK/PD modeling to select the PAC 200mg BID regimen in pts with MF treated in the PERSIST-2 trial.

Methods: Pts with MF and baseline platelet count ≤100,000/µL were randomly assigned 1:1:1 to PAC 400mg QD, PAC 200mg BID, or BID. Blood samples were collected from PAC-treated pts for PK and PD analysis at a prespecified subset of trial sites. Blood samples were collected on day 1 of week 1 (4 h post-dose), week 3 (pre-dose and 4 h post-dose), week 12 (pre-dose), and week 24 (pre-dose). At the remaining sites, blood samples were collected from PAC-treated pts for PK analysis only at weeks 12 and 24 (pre-dose).

Results: Blood samples were collected up to week 24 from 144 PAC-treated pts (78 BID, 64 QD). The PK of PAC was described by a 2-compartment model with first order absorption, first order elimination from the central compartment, and an absorption lag time. PAC QD was associated with higher Cmax and lower Cmin vs PAC BID (Table). Median PAC plasma concentrations during the PERSIST-2 trial were higher with QD and PAC unclassified vs PAC BID at steady-state (Cminss). At weeks 12 and 24 were higher with BID vs QD dosing by 10% and 15%, respectively. Also, median observed steady-state 4h-concentration at week 3 (coincides with Cmaxss) was 12% higher with QD vs BID dosing. In an exposure-response analysis, with QD or BID dosing, no trends were detected for a relationship between observed Cminss and death, cardiac death, hemorrhagic death, thrombocytopaenia (grade ≥2 or ≥3), anemia (grade ≥2 or ≥3), or gastrointestinal events (any grade, grade 2, or ≥3). Eleven (15%) and 13 (17%) PAC QD pts achieved SVR ≥35% and TSS reduction ≥50% at week 24, respectively, vs 16 (22%) and 24 (32%) PAC BID pts. Treatment with PAC BID but not QD showed a trend of increased SVR vs Cminss.

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, CYTOGENETICALLY CRITICAL FUSION IN MYELOID/MYELOID MALIGNANCIES OR 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 these fusions remains unknown. ZMYM2-FLT3 remains unexplained. We hypothesized that some MPN-eo cases may be driven by hitherto undetected cryptic TK fusion genes.

Aims: To screen cases with MPN-eo for TK fusion genes and evaluate the sig- nificance of any novel fusions

Methods: Poly(A) RNA extraction from MPN-eo cases, RNA-Seq library prepa- ration and 100bp paired-end sequencing was performed with multiplexing for a minimum of 75 million reads/sample using an Illumina HiSeq 2000. Bowtie, TopHat and TopHat-Fusion were used to align reads, resolve splice junctions, identify and filter potential TK fusion genes. Confirmation and screening of fusions was performed by RT-PCR and Sanger sequencing.

Results: 20 cases were 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, similar with 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) CYTOGENETICS was normal and FIP1L1-PDGFRα, KIT D816V and JAK2 V617F were all negative and no pathogenetically relevant mutations were identified by a myeloid NGS panel (28 genes). After 10 months, she progressed to myeloid blast phase. Because the disease was resistant to AML-induction chemotherapy (FLAG-Ilda), an allo- geneic PBSCT was performed after 10 months of a triple TKI (SUNITINIB, RICOSERTIB, AZD7473) treatment for refractory disease. As a consequence of chronic GVHD and septic shock, the patient died 6 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 CYTOGENETICS 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, leucocytes/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. These cases may represent a previously undetected TK gene fusion. Previous studies have identified a proportion of MPN cases and thus, although very rare, this fusion should be considered in the work up of MPN-eo cases. Due to their extensive diversity, we anticipate that RNAseq will become the method of choice to detect rare TK fusions.
E1345

COMPLETE HEMATOLOGIC AND CYTODYNAMIC RESPONSE IN A PATIENT WITH FIBROBLAST GROWTH FACTOR RECEPTOR 1 ACTIVATED MYELOPROLIFERATIVE NEOPLASM RECEIVING INCBO54828

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Background: Fibroblast Growth Factor Receptor (FGFR) inhibitors have demonstrated efficacy in solid tumors with FGFR pathway activation. INCBO54828, a novel, highly selective FGFR1, FGFR2, and FGFR3 inhibitor, is being assessed for the treatment of several advanced malignancies (AACR 2015; Abstract 771). 8p11 myeloproliferative syndrome is an aggressive myeloproliferative neoplasm (MPN) associated with FGFR1 translocation on chromosome 8p11.

Aims: To describe the characteristics of a patient with FGFR1 activated MPN who achieved a complete hematologic and cytodynamic response with INCBO54828 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 FGFR1/FGFR2/FGFR3 alteration (part 2). Had Eastern Cooperative Oncology Group performance status score ≤1 (parts 1 or 2 ≤2) and were refractory to prior therapy with no known effective standard therapy available to them. Patients received INCBO54828 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 a de novo translocated MPN diagnosis (currently the only patient with MPN enrolled in this trial), presented with abnormal white blood cell (WBC) count (eosinophilia, 15%; peripheral blood [PB] blasts, 4%) and abnormal platelet count (68 x109/L). The patient had prior therapy with hydroxyurea. Bone marrow (BM) biopsy at study entry showed 95% cellularity, 15% atypical megakaryocytes, (8,9)(11.2;q33) in 19 of 20 metaphases, and European Myelofibrosis Network grade MF-1. After 6 weeks of treatment with INCBO54828 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;q33) [3][4]/ 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: INCBO54828 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 INCBO54828 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 to be reproducible and adopted by the updated WHO 2016 classification.

Aims: In this study, we aim to evaluate any possible prognostic implications of this grading system in a series of patients with primary myelofibrosis.

Methods: The study involved 122 consecutive patients with primary myelofibrosis diagnosed between 1998 and 2015 at the Oncohematology Division of the Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico of Milan, for whom a bone marrow trephine biopsy ranging from 0 to 9 allows us to distinguish 88 (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 IBD in patients with MPN.

Methods: We used valid Danish national registries, covering more than 5 million individuals, and included all patients diagnosed with either essential thrombocythemia (ET), polycythemia vera (PV), myelofibrosis (MF), or unclassifiable myeloproliferative neoplasm (MPN-U) between 1994 and 2013. For each patient, 10 individually age- and sex-matched comparisons were included. Patients and comparisons were followed until first occurrence of any IBD diagnosis (ulcerative colitis or Crohn’s disease), death, emigration or end of 2013. Patients and comparisons with prior IBD were excluded from the analysis. Hazard ratios (HRs) between MPN patients and comparisons were estimated using Cox regression models, and used as measure of the relative risk.

Results: Of the 8,210 MPN patients, 80 individuals were diagnosed with IBD during the study period; including 37 ET patients, 28 PV patients, 1 MF patient and 14 MPN-U patients. During a total risk time of 45,241 years, the rate of IBD per 1000 person years at risk was 1.8 (95% confidence interval [95% CI]: 1.4-2.2) for the MPN patients. The corresponding rate for the 81,326 comparisons was 1.7 (95% CI: 1.6-2.0) per 1000 person years. The 10-year risks of IBD for MPN patients and comparisons were 0.8% (95% CI: 0.6-1.0) and 0.4% (95% CI: 0.4-0.5), respectively. The overall HR of IBD was 2.4 (95% CI: 2.1-2.9) for MPN patients, with HRs of 2.6 (95% CI: 2.1-3.2) for ulcerative colitis and 2.4 (95% CI: 1.7-3.4) for Crohn’s disease. The risk of IBD was increased 2 to 3 fold among ET, PV and MPN-U patients, with HRs of 2.8 (95% CI: 2.1-3.7) for ET patients, 2.1 (95% CI: 1.6-2.7) for PV patients and 2.2 (95% CI: 1.3-3.7) for MPN-U patients.

Summary/Conclusions: Patients with MPN are at increased risk of IBD compared to the general population. The absolute risks of IBD are low, but abdominal discomfort may in few patients be caused by underlying IBD.
ESSENTIAL THROMBOCYTHEMIA WITH AQUAGENIC PRURITUS: AN ENTITY WITH MORE AGGRESSIVE CLINICAL AND BIOLOGICAL PROFILE AT THE DIAGNOSIS AND A HIGH MORBIDITY DURING THE FOLLOW-UP

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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.

Aims: 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 Elopaeosis myeloprolétiffiées), 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 thrombocytopenic) vs ET without AP (p=0.04, 4x, ns). Concerning the occurrence of thrombotic events (arterial or venous) at diagnosis, no significant difference between patients with or without AP was found. In contrast, the presence of AP induced an increase of thrombotic events during the follow-up (30.9 vs 17.2%, p=0.03). But surprisingly, these events appeared in the delayed timing. The arterial/venous rate of thrombotic events was also different with 50/50 vs 25/75. 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 noticed 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 long 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 a higher risk of thromboses and phenotypical evolutions than ET without AP. Despite that these patients have a higher overall survival. So, the presence of AP in ET with characteristic patients with high risk of morbidity (thromboses, phenotypic 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 thrombocytosis in MPN. Although it is known that in thrombocythemia 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 purpose of this study was to determine the diagnosis of MPN due to the discovery of driver mutations (JAK2, careticulin 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 patients with MPN diagnosed in our centre between 1993 and 2015 were studied. The median age was 49 years, with 19 patients older than 60 years. 83% were female and 17% were male. The diagnosis was initially carried out based on the WHO criteria 2008 and subsequently reviewed the medical records with the new criteria of 2016. A molecular study on peripheral blood cells was carried out using quantitative allel-specific PCR techniques for JAK2, qualitative for MPL (L515V mutation) and Sanger sequencing on exon 9 for careticulin. Type 1 mutation was considered at 52 bp deletion and type 2 at 5 bp insertion. In all patients, the goal of anagrelide therapy was to control thrombocytosis (platelet count below 600x10^9/L) with dosage within the range of efficacy and safety recommended in the datasheet. The results were analysed with the statistical software SPSS vs 15.0

Results: 80.5% of the patients were diagnosed with ET, 12.5% of PV, 3.5% of myelofibrosis and 3.0% of unclassifiable MPN. 59% of the patients had a V617F JAK2 mutation, with allele load higher than 20% in 47.5% of the cases. 28.5% presented mutation in careticulin; 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 careticulin and the other 50% of the mutation V617F for JAK2 with allele load higher than 20%. 26% of the patients received daily dose of 1mg, being 70% positive for the mutation V617F/JAK2 with allele load lower than 20%, although there were no statistically significant differences between the groups according to the mutational profile. 16% of patients dis- continued 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 careticulin or JAK2 V617F allele load higher than 20% and patients with lower allele load having greater sensitivity to the drug, with no statistically significant differences. It is possible that the first situation is associated with a greater pre-mitotic deregulation in the megakaryocyte where the drug does not interfere whereas the second one could be related to anagrelide interference through the JAK2 pathway in post mitotic maturation although larg- er exploratory studies are required.

The delayed diagnosis of Philadelphia-negative myeloproliferative neoplasms (MPN) is common and results in a high incidence of potentially preventable thrombotic complications

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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 mortality. 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) abnormality 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 diagnosis delay 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 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 haemorrhagic events. Earlier recognition of FBC abnormalities consistent with MPN and earlier referral to specialist haematological management would, with earlier intervention, would be expected to prevent many thrombo-haemorrhagic 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 (B) is a possible second-line treatment, but conventional schedule

Reference: haematologica | 2017; 102(s2) | 555
(starting dose of 14mg/week up to obtain the complete haematological response (CHR) 
is associated with high risk of leukemic transformation and second malignancies. 
Aims: We analysed efficacy, toxicity, risk of Myelofibrosis (MF) and leukemic 
evolution in 31 of 352 ET pts collected in our database, treated with an alter-
native long-term schedule of BU, defined by low-starting dose (4-6mg/week) 
up to CHR (evaluated according to ELN response criteria), followed by dose 
de-escalation overtime.
Methods: Non parametric tests, such as Mann-Whitney, Pearson Chi-square 
and Fischer’s exact tests, were used for statistical analysis of continuous and 
categorical variables. Survival curves were calculated by Kaplan-Meier method 
and compared with Log-rank (Mantel-Cox) test.
Results: 27/31 pts were evaluable for analysis (8 male, 19 female). Median 
age at diagnosis and at BU start were 71,3 and 79 years (yrs) respectively. We 
found these driver mutations: JAK2V617F in 15 pts (55,6%), Calreticulin in 8 
pts (29,6%) and MPL in 1 patient (3,7%); 3 pts (11,1%) were triple negative. 
IPSET score at diagnosis was low-intermediate in 17 (63%) and high in 10 
(37%) pts. 26 pts started BU as 2nd line treatment: 11 (42,3%) were intolerant 
and 15 (57,7%) were resistant to HU respectively. Only one received BU as 1st 
line treatment. They received BU for a median time of 47,67 months (range: 
1,48 – 94,42). The median cumulative BU dose was 453mg (range: 32-1032). 
25/27 pts (92,6%) obtained CHR, after a median time of 191 days. 6 pts (22,2%) 
presented hematological (5) and extra-hematological (1, cutaneous) side 
effects. Overall, 12 pts (44,4%) stopped BU: 4 for hematological toxicity, 4 for 
disease progression, 2 for drug intolerance/resistance; the remaining 2 not for 
drug-related side effects. After a median follow-up of 9,74 yrs (range: 1,82- 
27,05), 9 (33,3%) and 2 (7,4%) pts presented MF evolution and leukemic 
transformation respectively. The MF-free-survival (MFS) was 48,8% at 15 yrs and 
appears to be significantly lower than the entire series of ET pts (77,4% at 15 yrs; 
p=0,002; figure 1). Median MFS was 12,7 yrs for pts treated with BU, 
whereas it was not reached at 15 yrs in the entire series of ET. There were no 
statistically significant differences in principal hematological and clinical features 
whereas it was not reached at 15 yrs in the entire series of ET pts. There were no 
statistically significant differences in principal hematological and clinical features 
except for ET patients without thrombosis. There was no difference in 
the frequency and type of 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 (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 in ET, PV and PMF patients with 
high heterogeneity in V617F allele burden between all studied groups regard-
less of the occurrence of thrombosis.

Figure 1.

Summary/Conclusions: Our experience with an alternative long-term and 
low-dose BU administration is safe and effective in elderly patients with ET. 
92,6% of them obtained CHR, with acceptable hematological and extra-hema-
tological toxicity. We noticed a high rate of MF evolution with respect to global 
ET population, while the risk of leukemic transformation seems to be limited, 
considering that these pts were elderly and previously treated. Predictive factors 
for MF evolution should be analysed and confirmed in larger series.

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(-) myelo-
proliferative neoplasms and the reasons for that are still under investigation.
**Non-Hodgkin & Hodgkin lymphoma - Biology**

**E1353**

**PROTECTION AGAINST DEVELOPMENT OF B CELL LYMPHOMA BY TETRASPANIN CD37**

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**Background:** B cell non-Hodgkin lymphoma, worldwide the most common hematological malignancy, remains a clinical problem. The molecular events leading to B cell lymphoma are only partially defined. CD37 is a member of the tetraspanin superfamily that is highly expressed on mature B cells and is required for optimal GC function and long-lived antibody production.

**Aims:** We investigated the function of tetraspanin CD37 in the development of B cell lymphoma.

**Methods:** A combination of studies was performed in mouse models (CD37/IL-6-deficient mice), and studies of DLBCL patient material using biochemical, immunological, genetic and microscopical approaches.

**Results:** We provide evidence that deficiency of CD37 induces the development of B cell lymphoma in vivo. Cd37-deficient mice develop germinal center-derived B cell lymphoma in lymph nodes and spleen with higher incidence than Bcl2-transgenic mice. We discovered that CD37 interacts with SOCS3, and when absent drives tumor development through constitutive activation of the IL-6 signaling pathway. The importance of the IL-6 pathway was confirmed by investigating Cd37xIl6 double knock-out strains that were fully protected against lymphoma development. Our unpublished data shows discovery of inactivating CD37 mutations in patients with DLBCL. Importantly, loss of CD37 on neoplastic cells in patients with diffuse large B cell lymphoma (DLBCL) is directly correlated with activation of the IL-6 signaling pathway and with worse progression-free and overall survival.

**Summary/Conclusions:** Together, this study identifies tetraspanin CD37 as a novel tumor suppressor that directly protects against B cell lymphomagenesis, and provides a strong rationale for blocking the IL-6 pathway in patients with CD37-negative B cell malignancies as therapeutic intervention.

**E1354**

**COMITANT DUAL ABLATION OF BLIMP1 AND P53 IN B-CELLS AS A NOVEL IN VIVO MODEL FOR HIGH-GRADE B-CELL LYMPHOMA**


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**Background:** B-Lymphocyte-Induced Maturation Protein-1 (BLIMP1)- and p53-inactivation contributes to the pathogenetics of a wide spectrum of malignancies, including diffuse large B-cell lymphomas. Nevertheless, there is lack of in vivo models that may be used for a better understanding of the biology and genomics of high-grade B-cell lymphomas characterized by dual loss of both BLIMP1- and p53.

**Aims:** To develop and characterize a transgenic mouse model of BLIMP1-deficient and p53-deficient B cells (1) To establish a new in vivo model that mimics human ABC-DLBCL phenotype.

**Methods:** Cre recombinase under the control of CD19 promoter (C57BL/6 Cd19CreCre) mice were crossed with either C57BL/6 BLIMPflox/flox or C57BL/6 Cre recombinase under the control of CD19 promoter (C57BL/6 Cd19Cre/p53flox/flox) and non-expressing the Cre recombinase, C57BL/6 Cd19Cre/p53flox/flox, to achieve dual deletion of BLIMP and p53 in B cells (Cd19CreCd19/Bl-/p53-deficient mice). Transgenic experimental mice (Cd19/Bl-/p53-) where characterized for clonal B cell infiltration using immunohistochemistry, flow cytometry, Southern Blotting, whole exome sequencing. MTT assay was used to test BTK-inhibitor-dependent cytotoxicity using CD19/Bl-/p53-deficient B220 cells.

**Results:** CD19/Bl-/p53- mice presented with diffuse lymphadenomegaly, splenomegaly, hepatomegaly (100%, 90.3% and 77.4%, respectively). Other clinical manifestations included presence of ascites and hind limb paralysis (12.9% and 19.3%, respectively). The CD19/Bl-/p53- showed increased overall survival compared to Bl-/p53- non-expressing the CD19/Cre recombinase, CD19/p53flox/flox or CD19/Bl- (363, 469.5, 460.5 and 770 days, respectively). H.E. staining of CD19/Bl-/p53--derived lymph node showed, a nodal architecture with a monomorphic population of large sized atypical lymphoid cells, multiple birefringent calcium deposits, paracentrally situated nucleoli. A "starry sky" pattern was also observed. Features were compatible with a high-grade lymphomas. IHC analysis confirmed positivity for B220 staining (TdT, Bcl6, CD138 and CD4, CD8 negative). Tumors were confirmed to be B220+lgM+ with either lgk- or lgdelta-lambda-restriction as demonstrated by flow cytometry, and either monor- or biclonal B cell populations were demonstrated by Southern blotting. Viral DNA was detected from B220+ selected cells obtained from pathological lymph nodes of CD19/Bl-/p53- mice and identified 143 SVNs. Non-synonymous somatic mutations were mapped on genes involved in the regulation of focal adhesion, PDGF signaling, p53-downstream pathway, and lipid protein metabolism. B220+ cells selected from CD19/Bl-/p53- derived lymph node were implanted s.c. into recipient SCID/Bg mice, and presented with 100% engraftment, with a monomorphic lymphoid infiltration of B220+ and IgM+ cells. B220 positive cells were selected from the s.c. tumor and intravenous injected into recipient SCID/Bg (n: 10) and BL6 mice (n: 10). Engraftment was demonstrated in all three groups where hepatomegaly and splenomegaly were observed. Infiltration of B220+ cells was documented within bone marrow, liver and spleen. Finally, we found that B220+ cells selected from lymph node hetero-thesized from CD19/Bl-/p53- mice were sensitive to ibrutinib.

**Summary/Conclusions:** Double inactivation of p53 and BLIMP in B-cells supports the concept of a novel in vivo model for dual deletion that recapitulates what seen in patients with ABC-DLBCL driven by BLIMP1-p53 dual loss-induced C-Myc expression.
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 germinal center-derived DLBCL (GC-DLBCL), through stabilization of the GC-DLBCL oncogene Bcl-6.

Aims: Here, we have explored if HSP110 could also be involved in the survival of the most aggressive form of DLBCL.

Methods: The study was performed with ABC-DLBC patient samples and several cell lines. Silencing specific for HSP110 was achieved through a lentiviral vector designed to inject 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 tumor sections and transcriptional analysis of ABC-DLBC patient tumors. Furthermore, shRNA silencing of HSP110 decreases the survival of several ABC-DLBCL cell lines, and downregulates the expression of pro-survival factors such as Bcl2 and Bcl-XL. SirRNA silencing of HSP110 abrogates NF-κb signaling, which is the major oncogenic pathway in ABC-DLBCL cell lines. In accord with these results, over-expression of HSP110 in DLBCL and non-DLBCL cell lines increases NF-κb signaling, indicating a tight interplay between HSP110 and the NF-kB pathway. Using immune-precipitation in DLBCL cell lines and Duolink™ assays, we identified an in vitro and in cellulo interaction between HSP110 and Myd88, a critical protein of the NF-kB pathway that bears an activated mutation in many ABC-DLBC patients and that is responsible for lymphoma aggressiveness. Finally, we demonstrate that HSP110 stabilizes the wild type as well as the mutated form of Myd88, therefore facilitating the chronic NF-κb pathway activation in those cells.

Summary/Conclusions: In conclusion, we identified HSP110 as a regulator of NF-κb 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.

Figure 1.
Aims: Here, we aim to recapitulate MCL in a mouse model of hematopoietic-specific overexpression of cyclin D2. Next, we want to use this preclinical mouse model to evaluate novel therapeutic strategies for the treatment of MCL.

Methods: To evaluate if cyclin D2 could act as a bona fide oncogene in the pathogenesis of MCL, we developed a conditional R26-driven Ccn2d2 overexpression mouse model. For this, we used a targeting vector that contained a floxed stop cassette followed by the Ccn2d2 gene and a EGFP/luciferase reporter, which was subsequently targeted in mESCs using recombinase-mediated cassette exchange (RMCE).

Results: The resulting R26-Ccn2d2 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. Using the (E1B1) viral oncogene, we confirmed the formation of 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 strategy holds a considerable potential in aggressive B-cell tumors with a high rate of protein synthesis, HDAC6 inhibitors - are currently being tested in Phase II/III clinical trials. The results of preclinical studies show the increased efficacy of the combination of HDAC6 inhibitors with proteasome inhibitors in inducing stress-related cell death. The results of our studies show that HDAC6 inhibition in non-toxic concentrations significantly increases CD20 level on a protein level.

Aim: Our aim of the study was to elucidate the mechanism of the regulation of CD20 expression by HDAC6.

Methods: We used qRT-PCR and Dual Luciferase Assays in order to determine the influence of HDAC6 on CD20 transcription. We used pulse-chase using assays widely used with translation inhibitors – cycloheximide and homoharringtonine.

In order to study the effect of HDAC6 inhibition on global as well as specific translation of CD20 after HDAC6 inhibition we studied the formation of stress granules (SG).

Results:

We used qRT-PCR and Dual Luciferase Assays in order to determine the influence of HDAC6 on CD20 transcription. We used pulse-chase using assays widely used with translation inhibitors – cycloheximide and homoharringtonine.

We show that HDAC6 inhibition regulates CD20 level without affecting its transcription. Moreover, we demonstrate that HDAC6 inhibition increases translation of CD20 mRNA on polysomes without affecting general synthesis of novel proteins in the cell. However, our experiments suggest that the transcribed translation of CD20 mRNA is not a result of resumed translation of mRNPs, but a result of its translation.

Summary/Conclusions: Our study shows a new mechanism of the regulation of CD20 expression by increasing its translation. Moreover, we demonstrate a new role of HDAC6 protein. This finding has a potential clinical application, as HDAC6 inhibitor are being widely tested in different hematological malignancies.
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

CLINICOBILOGICAL FEATURES OF B-CELL NEOPLASMS WITH CDK6 TRANSLOCATIONS: FREQUENT ASSOCIATION WITH MARGINAL-ZONE LYMPHOMA, CONTIGUOUS OF PROLYMPHOCYCTIC 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;14)(q21;q11), leading to juxtaposition of CDK6 gene with IGH, IGK or IGL locus respectively.

Aims: The Groupe Francophone de Cytophysiologie Hématologique (GFCH) collected 35 chronic B-cell disorders with CDK6 translocation in order to document the clinico-biological 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 IGH or TRAD and CDK6 rearrangements, and recurrent abnormalities frequent in SMZL and CLL (trisomy 3, 12, deletions of 13q14 and 7q22/7q36 loci).

Results: 9/35 (26%) K was complex in 18/35 (51%). The most frequent additional abnormalities were: del17p (TP53) (51%), del13q (41%), del16p (23%), trisomy 18 (22%), trisomy 19q (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 del17p. 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 L625P was detected in 2/22 patients. 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 duration to first treatment of 13 months. In our series, the CDK6+ MZL 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 (1/13). 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 are rare, and therefore, we only observed 1 case involving the T-cell receptor (TCR) locus, which is a rare event in a B neoplasm. Finally, it is intriguing that this abnormality involves almost exclusively the IKGK locus, and not the other IGH loci, especially IGH which is usually the most frequently rearranged.

E1362

PRIMARY CUTANEOUS DIFFUSE LARGE B-CELL LYMPHOMA, LEG TYPE, EXPRESS STEREOYPEOED B-CELL RECEPTORS WITH UNIQUE NONSYNONYMOUSLY MUTATED CONSTANT REGIONS

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Background: Primary Cutaneous Diffuse Large B-Cell Lymphoma, Leg Type (DLBCL, LT) is a rare and aggressive neoplasm with a primary cutaneous presentation that shares genetic and phenotypic characteristics with DLBCL of activated B-cell subtype (ABC-DLBCL). Although receptor stereotypes have been observed, the role of the B-cell receptor (BCR) in DLBCL, LT is largely unknown. Previous studies on small cohorts suggested that DLBCL, LT expresses 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 lymphoma were obtained from NCBI publicly available datasets and collaborators. VDJ/VJ rearrangements and IGH constant regions were Sanger sequenced for all cases and two granulocyte controls. Lymphoma-derived clonal BCR were tested for autonomous signalling activity in the murine TKO pre-B-cell system (Dührren-von Minden, Nature 2012).

Results: RNAseq analysis demonstrated an IGH isotype in eight of all VJ-kappa and VDJ gamma cases. B-cell specific (BCR, TR) on a mouse constant region backbone did not induce antigen-independent calcium flux in TKO cells upon induction of functional pathway of the BCR signalling cascade by tamofoexin.

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 IGHM backbone. The pathogenic potential of the novel constant region mutations for BCR activity in DLBCL, LT warrants further functional studies.
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 sh-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 forma-
tion in EµMyc Nr4a1+/+ (n=134), EµMyc Nr4a1 -/- (n=54) and EµMyc Nr4a1 +/-(n=59), respectively. For the PCR shRNA screen selected tumor specimens from wt and EµMyc mice with (n=14) and without (n=17) Nr4a1 loss were used. For investi-
gation 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, the driver-function of Nr4a1 in lymphomagenesis at the premalignant stage was investigated by using apoptotic assays and by carrying out transplantsations of tumor cells into wt recipients.

Results: EµMyc Nr4a1 -/- mice showed decreased survival with a median of 92 days compared to Nr4a1 +/+ mice with median survival of 123 days (p=0.001) and tumors developed faster with a median of 45 days for EµMyc Nr4a1 -/-, vs 107 days for EµMyc Nr4a1 +/+; p<0.001. Both, survival (median=101 days; p=0.037) and tumor formation (median=66 days, p=0.001) gave intermediate values for EµMyc Nr4a1 -/- mice. Furthermore, EµMyc Nr4a1 +/ - mice suffered less frequently from EµMyc Nr4a1 -/- mice, whereas apoptosis was significantly diminished in EµMyc Nr4a1 -/- mice. In vivo, the driver-function of Nr4a1 in lymphomagenesis at the premalignant stage, whereas apoptosis was significantly diminished in EµMyc Nr4a1 -/- mice. Kaplan Meier analysis was used for monitoring survival and tumor formation, and FACS analysis for analysis of bone marrow, spleen and tumor, respectively.

Summary/Conclusions: Our results clearly demonstrate the influence of Nr4a1 loss on tumor formation and consequently survival in a Myc-driven model of lymphomagenesis. Importantly, Nr4a1 loss seems to impact cell death early in B cell development, even ahead of malignant transformation. Additionally, Nr4a1 seems to be involved in driving immune responses towards an anti-inflamma-
tory, tolerogenic phenotype, thereby facilitating tumor growth and in altering the tumor environment. Collectively, these data underpin the tumor suppressive function of Nr4a1 in aggressive lymphomas.

E1365
MUTATIONAL PROFILING OF HODGKIN- AND REED-STERNBERG CELLS (HRSC) OF CLASSICAL HODGKIN LYMPHOMA (CHL) ENRICHED FROM ARCHIVAL FORMALIN-FIXED AND PARAFFIN-EMBEDDED TISSUE SAMPLES
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Background: ChL can be cured in the majority of cases. However, ~10–20% patients die of the lymphoma after relapse or progressive disease. There are unmet needs for understanding the mechanisms that cause ChL relapses, for development of new prognostic/predictive markers and effective targeted ther-
apies. Comprehensive genetic characterization and advance in understanding of the disease pathogenesis of CHL are indispensable to meet those needs. How-
ever, genetic information on CHL is still scarce mainly due to difficulties of iso-
lation 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 usability is limited because of poor DNA quality and difficulty to enrich 
neoplastic cells. Therefore, new enrichment techniques are necessary to enable larger 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 infor-
mation 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-assist-
ed sorting of cell nuclei (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 genes. Mutations of STAT6, PIM1, SOCS1, KMT2D occurred in at least 18% (2/11) of cases. Additionally, 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 demon-
strates that mutational profiling of 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

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Madrid, Spain, June 22 – 25, 2017
OUR data highlighted in each patient persistent gene modifications.

**Methods:** We characterized the spontaneous haematopoietic tumours 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. Experiments were performed in accordance with the national animal experimentation guidelines.

**Results:** A dominant miRNA signature consisting of 103 miRNAs was identified in STAT1-deficient mouse models.

**Summary/Conclusions:** Our data indicates that miRNA signatures might be involved in the development of STAT1-deficient lymphoma.

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**LACK OF STAT1 PREDISPOSES TO A DIFFUSE LARGE B-CELL LYMPHOMA-LIKE DISEASE**


**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.

**Aims:** To address the genetic heterogeneity in MCL in paired patient samples at diagnosis and relapse.

**Methods:** Highly pure malignant B-cell populations were isolated using fluorescence-activated cell sorting in four patients diagnosed with MCL. In addition, T-cells were sorted from the same patients as paired non-malignant control samples. Moreover, we performed on both the malignant B-cell population and paired T-cells (13 samples in total). Mutations were detected in parallel with CLC Biomedical Workbench 2.5 (Qiagen) and MuTect 1.04 (Broad Institute) (coverage ≥ 20, population allele frequency < 0.01) and evaluated against the COSMIC (Wellcome Trust Sanger Institute), dbSNP and PubMed databases. Exonction from informed consent was approved by the National Ethical Committee.

**Results:** Our data highlighted in each patient persistent gene modifications between diagnosis and relapse. We confirmed gene mutations already well-known in B-cell malignancies (e.g. TP53, NOTCH1 and MYD88). Interestingly, aberrations not previously described in the COSMIC database were observed with high allele frequency both at diagnosis and at relapse. This included genes in B-cell signaling (e.g. transcriptional repressor SPEN associated to NOTCH pathway regulation and blockade of the precursor B-cell differentiation), inflammatory response (e.g. JRG1), genes found in invasive carcinoma (e.g. integrin β4 subunit) and B PLAT monoclonal expression which were driven by somatic mutations or hit in putative drivers, new gene modifications as well as loss of previous ones could be observed at relapse.

**Summary/Conclusions:** Our work shows examples of molecular progression from diagnosis to relapse in MCL and supports the heterogenic nature and genetic complexity of this disease. We confirm mutations in genes already known as involved in the disease, and to identify new signaling pathways with genes which could be targeted by new treatments.

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**Molecular Heterogeneity of Mantle Cell Lymphoma**


**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.

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**Summary/Conclusions:** Our work shows examples of molecular progression from diagnosis to relapse in MCL and supports the heterogenic nature and genetic complexity of this disease. We confirm mutations in genes already known as involved in the disease, and to identify new signaling pathways with genes which could be targeted by new treatments.
imential conditions we found that a number of the captured genes corresponded to experimentally validated targets of mirR-155. Crucially, oncotherapy 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 tool genes. 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**

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**Background:** Daratumumab (DARA) is a first-in-class human monoclonal antibody that targets the CD38 epitope and is approved for the treatment of relapsed/refractory (R/R) multiple myeloma (MM) patients. DARA is currently being evaluated in phase II clinical trials as monotherapy in patients with R/R Mantle Cell Lymphoma (MCL), Follicular Lymphoma (FL) and Diffuse Large B-Cell Lymphoma (DLBCL). DARA inhibits cell growth through high-affinity, neo-antibody-mediated mechanisms in MM, including Antibody-Dependent Cellular Cytotoxicity (ADCC), Complement-Dependent Cytotoxicity (CDC) (de Weers M. J Immunol, 2011) and Antibody-Dependent Cellular Phagocytosis (ADCP) (Ovredijk MB. Mabs, 2015). In Chronic Lymphocytic Leukemia (CLL), DARA induces killing mainly via ADCC and ADCP (Matas-Céspedes A. Clin Cancer Res, 2016). Furthermore, Immunomodulatory effects (Krijnck J. Blood, 2016) and modulation of the enzymatic activity of CD38 (Lamerts van Bueren J. Immunol, 2011) have been described to contribute to its antitumor activity.

**Aims:** To evaluate the activity of DARA on MCL and FL cells as monotherapy and in combination with current therapies, both in vitro and in vivo.

**Methods:** ADCC, CDC and ADCP activities were assessed by calcein release or flow cytometry. Penetration of DARA was analyzed in a 3D model by Selective Plane Illumination Microscopy (SPIM). Molecules per cell were analyzed using Qifikit and flow cytometry.

In vivo activity was assessed in prophylactic and therapeutic set ups using SCID mice subcutaneously (sc) or intravenously (iv) injected with 1x10^7 of MCL or FL cells. Mice were treated (human IgG control or DARA) with two different schedules: prophylactic (3 doses of 10mg/kg one dose per week) or therapeutic (20/10/10/10mg/kg, one dose per week). For the combination regimens in FL, sc injected SCID mice were treated following the therapeutic schedule in combination with Rituximab (20/10/10/10mg/kg, one dose per week) and/or CHOP (initial unique dose).

**Results:** DARA (0.0001-1µg/mL) induced ADCC in a dose-response manner on MCL (n=6) and FL (n=4) cell lines in the presence of PBMCs in vitro. Moreover, DARA induced significant levels of ADCP at 1µg/mL on MCL (n=6) and FL (n=4) cell lines in the presence of murine macrophages in vitro. However, DARA did not induce significant CDC in any of these models due to a high expression of the complement inhibitors CD46, CD55 and CD59, and insufficient number of CD38 molecules per cell. In a 3D model of FL, SPIM analysis revealed a maximum penetration of DARAA at 1µg/mL after 48h of treatment. We tested DARA activity in vivo in two different mouse models (sc and iv) of MCL and FL. In the prophylactic setting, DARA completely prevented the outgrowth and induced tumor regression of MCL (n=6) and FL (n=6) subcutaneous tumors. In the therapeutic setting, DARA significantly increased the survival of mice and reduced organ infiltration of tumor cells both in the MCL (n=10) and in the FL (n=10) systemic xenograft models. In addition, the combination of DARA with Rituximab/CHOP regimen in FL resulted in a synergistic reduction of tumor growth (n=7-10).

**Summary/Conclusions:** DARA shows encouraging cytotoxic activity in MCL and FL cells in culture as well as in vivo and in combination with current approved therapies. These results warrant further studies of DARA in the clinical setting for these conditions.

**E1370**

**ECTONUCLEOTIDASES CD39/CD73 ARE HIGHLY EXPRESSED ON ATLL CELLS AND RESPONSIBLE FOR GENERATING AMP/ADENOSINE**

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**Background:** Adult T-cell leukemia/lymphoma (ATLL) is a mature T-cell neoplasm, linked to the human T-cell lymphotropic virus, HTLV-1. Patients with ATLL are often at the risk of opportunistic infections. It might be possible that this immunocompromised state could be induced by the function of ATLL cells having similar phenotypes with regulatory T cells (Tregs). However, difficulties of in vitro studies using primary tumor cells have hampered the progress of ATLL research, and it is still controversial whether ATLL tumor cells have the immunosuppressive characteristics.

**Aims:** In this study, we analyzed the roles of molecules expressed in ATLL cells associated with immunosuppressive functions of Tregs.

**Methods:** The protocol of this study was approved by the Investigational Review Board of Osaka University Hospital. Peripheral blood mononuclear cells (PBMCs) were collected from 8 asymptomatic HTLV-1 carriers and 20 ATLL patients (3 with smoldering type, 5 with chronic type, and 12 with acute type) after getting informed consent. PBMCs from 3 ATLL patients were separated into CD4+CD7-CADM1+ ATLL 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 ATLL patients or carriers or each type of ATLL patients.

**Results:** We compared whole transcriptome of ATLL cells and normal CD4+ cells. Bioinformative analyses showed that many genes associated with immunosuppressive functions of Tregs were elevated or downregulated in ATLL cells. Among these genes we focused on CD39, CD73 and CD26, because recently it has been reported that extracellular adenosine, which is catalyzed by CD39, expressed in human Tregs, and CD73, expressed in murine but not in human Tregs, has strong anti-inflammatory function and plays major role in Treg-mediated immunosuppression. Therefore, we investigated the expression of CD39 and CD73 in ATLL cell lines and primary tumor cells. We found that all of 4 ATLL cell lines expressed CD39, but not CD73 as just human effector Tregs. In contrast, the expression patterns of CD39 in 20 ATLL patients were various (Table) and interestingly, some ATLL tumor cells express CD73. Also in asymptomatic carriers, we could detect CD39 and/or CD73 positive on 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 ATLL cells was assessed. Extracellular ATP is converted into AMP by CD39. As expected, CD39+ ATLL cells converted significantly more ATP than CD39- ATLL cells, which was 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 AMP was converted to adenosine by CD73+ ATL cells, indicating that the aberrant expression of CD73 could not efficiently increase adenosine synthesis.

**Table 1.**

**Summary/Conclusions:** In this study, we showed that about two thirds of ATLL samples were CD39+/CD26+ just as effector Tregs and have comparable level of ATPase activity as Tregs, which are expected to play some immunosuppressive function in ATL patients. Recently it is also reported that in exhausted CD8+ T cells in cancer patients, CD39 is co-expressed with PD-1. CD39 expression in ATLL cells may also have some roles in immunosuppression and thus in the escape from anti-tumor immunity.

**E1371**

**Abstract withdrawn.**

**E1372**

**ACTIVATION OF SYK TYROSINE KINASE INHIBITORS PLAYS A ROLE IN RESISTANCE AGAINST THE SELECTIVE BTK INHIBITOR ONO/GS-4059 IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL)**

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**Background:** Activation of syk tyrosine kinase is a potential therapeutic target in diffuse large B cell lymphoma (DLBCL). Syk is a tyrosine kinase that is activated downstream of B-cell receptor (BCR) through BTK. Syk activation contributes to malignant transformation and resistance to BTK inhibitors. Syk activation is also associated with chemoresistance and monoclonal antibody refractoriness.

**Objectives:** The aim of this study was to investigate the role of Syk in the resistance to BTK inhibitors in DLBCL cell lines and patient samples.

**Methods:** We performed dose-response analysis of BTK inhibitors (ONO/GS-4059 and ibrutinib) on DLBCL cell lines and patient samples. We also performed Western blotting and immunohistochemistry to detect Syk expression.

**Results:** Our results showed that Syk activation is associated with resistance to BTK inhibitors. Syk expression was significantly higher in resistant cell lines compared to sensitive cell lines. Syk inhibition potentiated the effects of BTK inhibitors, indicating that Syk is a potential therapeutic target in DLBCL.

**Conclusion:** Syk is a potential target for the treatment of DLBCL. Syk inhibition potentiates the effects of BTK inhibitors, suggesting that Syk inhibition could be used for the treatment of DLBCL.

**Acknowledgment:** This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number 17H02348.
and proliferation of several B cell malignancies. BTK is a key regulator of this pathway. In a preliminary clinical study using the selective BTK inhibitor ONO-GS-4059 showed therapeutic activity in relapsed/refractory DLBCL of the Activated B-cell phenotype (ABC-DLBC) (Walter et al Blood 127 pp141-2016). However, median treatment duration in ABC-DLBC was only 3 months due to progressive disease and development of resistance. Two acquired resistant mutations to R665W have been described with resultant mechanisms to BTK inhibition in CL 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: TMD8, an isogenic sensitive ABC-DLBC cell line TMD1 was cloned ONO-GS-4059 and bortezomib resistant TMD8 cell lines (TMD8RO and TMD8RI) were used for this study. TMD8RO has PCLy2 R665W while TMD8RI lacks both BTK C481S and PCLy2 R665W. Cell viability and apoptosis after compound treatment were assessed using Cell titer Glo assay and Annexin V / PI staining. Western Blot analysis was performed on both cell lines to determine expression of immunoreceptor were assessed by immunoblot and Flow cytometry. The mutational status of BTK and PCLy2 in TMD8 was determined by Sanger sequencing.

Results: ONO-GS-4059 induced apoptosis in TMD8 with nanomolar concentrations with apoptosis observed in >80% of cells. Although ONO-GS-4059 induced rapid reduction in ERK and AKT activation, induction 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 C22, which negatively regulate BCR signaling, was decreased in these cells. The combination of ONO-GS-4059 and GS-9973 restored sensitivity to ONO-GS-4059 and induced synergistic apoptosis in both resistance cell lines.

Summary/Conclusions: These data show that SYK is highly activated through increased sIgM expression and/or downregulated CDS and C22 following BTO treatment. The combination treatment of ONO-GS-4059 and SYK inhibitor may combine not only the development but also the maintenance of resistance to BTK inhibitor. The combination of ONO-GS-4059 with SYK inhibitor is therefore a rational strategy for preventing and overcoming BTK inhibitor resistances.

E1373

STRO-001, A NOVEL ANTI-CD74 ANTIBODY DRUG CONJUGATE (ADC) FOR TREATMENT OF B-CELL NON-HODGKIN’S LYMPHOMA (NHL)

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Background: CD74 is a type II transmembrane glycoprotein involved in the formation and transport of MHC class II protein. CD74 is rapidly internalized and highly expressed in many B-cell malignancies with limited expression in normal tissues (Stein R. et al., CCR 2007). STRO-001 is a novel CD74-targeting ADC (Biotinylated SP7219) conjugated to a non-cleavable dibenzocyclooctyne (DBCO)-maytansinoid linker-warhead. Highly efficient site-specific conjugation enabled by Sutro’s cell-free antibody production and click chemistry produced a well-defined homogeneous ADC with a potency

Aims: The aim of this study was to investigate the therapeutic potential of STRO-001 in non-Hodgkin’s lymphoma (NHL) cell lines and xenografts. A dose-escalating exploratory toxicity study was also conducted in cynomolgus monkeys.

Methods: Biotinylated SP7219 was used for immunochemistry (IHC). DBCO-Alexa647-conjugated SP7219 and flow cytometry were used for detection and quantification of CD74 expression on NHL cell lines and B-cells from normal human donors. STRO-001 was used to determine the EC50 and percent span of killing in NHL cell lines. The anti-tumor activity of STRO-001 in SCID mice xenografted with NHL xenografts was evaluated in a xenograft model and in mice bearing NHL tumor cell xenografts was examined. STRO-001 was administered to cynomolgus monkeys in an exploratory dose-escalating study of clinical pharmacology, Pitie-Salpetriere Hospital, Paris, France

Background: Leptomeningeal dissemination (LD) is a relatively rare but often fatal complication of lymphomas, confirmed by the analysis of the cerebrospinal fluid (CSF). The diagnosis is suspected in case of neurological symptoms, parenchymal brain involvement detected with neuroimaging techniques and increased sIgM concentration in the CSF. Cytological examination (CM) is still considered as the “gold standard” but remains insufficently sensitive.

Aims: The aim of our study was to assess the benefit of more sensitive techniques, i.e. immunophenotyping by flow cytometry (FCM) and clonality by PCR. The detection of malignant cells in CSF was performed by FCM and PCR in a slow growing disseminated NHL xenograft model. Vehicle-treated animals developed advanced disease with palpable tumors and distended abdomens, with median survival of 81.3 days. In contrast, Mino xenografts treated with STRO-001 at 3mg/kg or 10mg/kg exhibited improved survival, with most animals healthy and disease free at the time of sacrifice 135 days post inoculation. STRO-001 demonstrated B-cell depletion in cynomolgus monkeys, confirming the intended pharmacodynamic effect. Myelosuppression was observed at the highest dose but there was no evidence of off-target toxicity.

Table 1.
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+ and/or PCR+ but CM-. A total of 699 samples showed similar results by FCM and PCR with presence (n=22) or absence (n=247) of lymphomatous cells whereas 25 samples were classified as suspicious by at least one technique. Eleven samples were FCM+ but PCR-. False negative (FN) PCR results can be explained in part by extensive somatic mutation in IG genes, preventing optimal detection of the VDJ+ venetoclax sensitive cell lines, 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 detection of the venetoclax sensitive cell lines). This may not systematically require intact cells. Most of the difficulties encountered with both methods are due to occult blood contamination and poor cellularity, leading to low-intensity clonal signals by PCR and inconsistent cluster of events with FCM. In addition discordant results between FCM and PCR might be explained by sampling heterogeneity. Considering these limitations, it seems highly advisable to choose the best suited method for the follow-up according to the results at diagnosis.

**Summary/Conclusions:** Our results suggest that a multimodal investigation using FCM and PCR is necessary for improved detection of leptomeningeal dissemination in 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.

E1375

THE SYK INHIBITOR R406 DRAMATICALLY INCREASES THE SENSITIVITY OF GCB AND ABC DLBL 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 (Davids MS et al, J Clin Oncol. 2017).

Aims: Investigate whether the SYK inhibitor R406 can increase sensitivity of DLBCL to venetoclax in vitro and to venetoclax.

**Methods:** The following cell lines were used: Ly1, Ly7, Ly18, DHL4, Toledo and BJAB (all GCB DLBCL) and U2932, DHL2, Ly3, Ly10, HBL1 and TMD8 (all ABC DLBCL). The percentage of apoptotic cells was determined by Annexin V/FITC staining and propidium iodide (PI) staining and flow cytometry analysis. Expression of BCL-2 family members was determined by immunoblotting or Q-RPQ-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 an additive effect was observed in the remaining cell lines (Ly4, Ly7, Toledo, BJAB, DHL2 and Ly3). Among these, only Toledo expressed similar levels of BCL-2 to that observed in the venetoclax sensitive cell lines whereas the levels of BCL-2 in the other cell lines were extremely low or undetectable. To understand the mechanisms how R406 increases the sensitivity of DLBCL cells to venetoclax, we evaluated changes in the expression of MCL-1 and other antiapoptotic BCL-2 family proteins that have been associated with venetoclax resistance. Five of the seven R406 + venetoclax sensitive cell lines (Ly1, DHL4, U2932, HBL1 and 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-2/L cl 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 proapoptotic BCL-2 family member that was recently shown to be induced by SYK inhibition in a subset of GCB DLBCLs (Chen L et al, Cancer Cell. 2013). A substantial increase in HRK expression (140-640%) was observed in 5 of the 7 R406 + venetoclax sensitive cell lines (Ly1, Ly18, DHL4, U2932 and TMD8).

Figure 1.

**Summary/Conclusions:** These data show that the SYK inhibitor R406 can significantly increase the sensitivity to venetoclax in the vast majority of BCL-2 positive DLBCL cell lines. The mechanisms of action require further investigation, but are likely to involve downregulation of MCL-1 and upregulation of HRK in a substantial proportion of cases.

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 (post-thymic) T-cell leukemia. Clonal TR gene rearrangements are detected in virtually all T-PLL by FCM or PCR from peripheral blood (PB) or bone marrow samples.

Aims: To compare the results of parallel TRB-based clonality analyses by FCM and NGS in T-PLL.

**Methods:** We investigated diagnostic PB leukocytes of 73 T-PLL patients with median lymphocytes at 66% (range 13-93; harboring T-cells at 97% (55-100)). FCM of surface (not intracellular) 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 (2x300bp + 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-2V- and J-region primers (van Dongen et al, Leukemia 2003). In the second PCR step, sequencing adaptors and sample-specific barcodes were added. Annotation of V, (D)- and J-regions of TRB sequences was done using ARResT/Interrogate (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 15-95%). 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 (2x300bp + v2) with a median coverage of 17,908 reads (range 1,125–41,193)/sample. In the first PCR TRB rearrangements were amplified using TRB BIOMED-2 V- and J-region primers (van Dongen et al, Leukemia 2003). In the second PCR step, sequencing adaptors and sample-specific barcodes were added. Annotation of V, (D)- and J-regions of TRB sequences was done using ARResT/Interrogate (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 15-95%). 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 clonotype detected by NGS corresponding to a bi-allelic TRB rearrangement with the second non-functional allele being preferentially identified by NGS. In 37/73 (51%) of cases no reaction with one of the 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 despite theoretical coverage. Of note, overall 10/73 T-PLL (14%) lacked surface TRαβ 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 of FC- or IFN-β-based clonality detection can best be explained by lost surface TR expression and the limited coverage of the VB antibody panel. NGS-based clonality analysis can overcome these limitations, because it detects virtually all TRV VB-JB rearrangements. On the contrary, NGS is more sensitive and therefore enables the detection of minor subclones, which has great appeal for MRD assessment. Nevertheless, flow cytometric VBJ spectratyping is a faster, cheaper, and less laborious alternative. It has the additional advantage of detecting the actual TR VB chain expression and of visualizing individual T-cell subsets for quantification of VB-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 sensitivity is mediated can identify new targets for therapy or predictive biomarkers of response. Using an in vitro model system we have identified the transcription factor IRF4 as a sensitive indicator for BTKi response in MCL cell lines and primary cells.

Aims: To identify molecules or pathways responsible for resistance to BTKi-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 continuously 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-cell receptor or CD40L stimulation, and their sensitivity or resistance to treatment was determined using flow cytometry to assess proliferation (Ki67), apoptosis (Annexin-V), or phosphorylation of BTK (pY223). Changes affecting downstream proteins were determined by protein expression or phosphorylation analysis (immunoblotting) and by mRNA expression (RT PCR). Following initial experiments the studies focussed on IRF4.

Results: Each MCL cell line showed basal phosphorylation of BTK (Y223) and its downstream effector molecule ERK1/2 (Y204/187); in each case phosphorylation was prevented by BTKi. Of the cell lines tested however, only REC-1 cells showed growth inhibition by BTKi (ibrutinib and acalabrutinib), demonstrating both dose-dependent apoptosis (p<0.01) and inhibition of proliferation. Further investigation showed that only the BTKi-sensitive REC-1 cell line downregulated IRF4 in response to BTKi; this downregulation was an early and specific response (mRNA downregulated after 4 hours, and protein expression after 8 hours). Furthermore in REC-1 cells with acquired partial resistance to BTKi, the downregulation of IRF4 was significantly less than in the parental cell line. Finally in vitro co-culture of REC-1 cells with CD40L prevented IRF4 downregulation thereby counteracting the effect of BTKi induced apoptosis. 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: IRF4 was downregulated in 6 samples from patients shown to be clinically responding to BTKi and was not downregulated in 1 refractory case. Treatment with BTKi might be a biomarker for BTKi-sensitivity in MCL, and that downregulation and protected the cells from BTKi-induced apoptosis. 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.

Figure 1.

Summary/Conclusions: This study reveals a novel pathway during myc-driven lymphomagenesis. We show that MYC deregulation imposes selective pressure in favor of clones with decreased expression of TPL2 kinase. This process seems to be advantageous for the malignant clone, since genetic ablation of TPL2 in the Eμ-myc mouse model accelerates MYC-induced lymphomagenesis likely by contributing to apoptosis resistance.

E1379
LIQUID BIOPSY: DECIPHERING A SIGNATURE OF CIRCULATING MICRONAS AS NOVEL NON-INVASIVE BIOMARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Up to 40% of Diffuse Large B-Cell Lymphoma (DLBCL) patients still experience treatment failure or disease relapse after conventional chemotherapy. Therefore, the search of novel non invasive biomarkers able to early identify these patients is warrant in order to offer a different therapeutic approach. Recently, bodily fluids have emerged as an important source of infor-

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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 means of a quantitative competitive RT-PCR. Pt enrolled in our study were treated with R-CHOP and sampled at diagnosis of the first 18 patients enrolled into the study.

Results: Our results showed that the expression level of serum miR-22 as well as let-7c/miR-99a/miR-125b cluster was significantly higher at diagnosis, in patients unresponsive to R-CHOP treatment when compared with responsive patients. On the contrary, miR-18 and miR-20 levels appeared to be not significantly associated to treatment response. In addition, a global expression profile of circulating miRNAs was evaluated in serum samples derived from a smaller cohort of patients (n=4) after first-line chemo-immunotherapy. Interestingly, we found a striking difference in miRNA modulation upon treatment between unresponsive and responsive patients. In particular, we found 31 miRNAs significantly modulated after R-CHOP in the group of responsive patients, including miR-22. In contrast, this miRNA subset did not show remarkable expression changes in unresponsive patients. Moreover, we performed a study interrogating The Cancer Genome Atlas (TCGA) database about miRNA expression levels in samples of DLBCL patients. We found that the only available data are relative to the miRNA expression levels in tumor tissue samples of 47 out of 58 DLBCL patients. Kaplan Meier method and log-rank test revealed a signature of 13 miRNAs with potential prognostic value. Among these we found that miR-22, also emerged as modulated in our genome-wide analysis, was linked to risk of disease recurrence.

Summary/Conclusions: These preliminary data suggest that the serum miR-22 as well as miR-99a/miR-125b cluster are of potential interest as non–invasive biomarkers to predict therapeutic response in DLBCL patients. Ongoing experiments in a wider cohort of patients are aimed to confirm these results and unveil potential miRNA signature with predictive value.

E1380

INTRACELLULAR CALCIUM AND METABOLISM HAVE CRITICAL ROLES IN DETERMINING ANTI-CD20 ANTIBODY EFFICACY IN DLBCL

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Background: Since the discovery and utilisation of the Type-I anti-CD20 antibody Rituximab, many have tried to enhance the efficacy of anti-CD20 antibodies in order to improve first-line treatment of B cell malignancies, leading to the discovery of anti-CD20 antibodies with different intrinsic properties. Understanding 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 function 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: 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 (Karposi222, Pfeffer, OCI-LY7 and HUDHL4). Following a 24-hour treatment with one of four anti-CD20 antibodies (Rituximab) and three Type-II anti-CD20 antibodies (BHH2, Obinutuzumab and Tositumomab), intracellular calcium concentration was quantified and visualised using imaging flow cytometry. Next, we used the XF Seahorse Mito Stress Test to reveal bioenergetic profiles of the cell lines following a 24-hour treatment with the same antibodies. We used Metformin (a mitochondrial inhibitor) and oxypHos (an activator of mitochondrial function). Metformin was also used to reduce the mitochondrial membrane potential (MOMP) across our panel of cell lines. We confirmed MOMP reduction by staining cells with JC-1, a chameleon dye used as an indicator of MOMP and analysed samples using flow cytometry. Under the same conditions, we conducted clonogenic survival assays to see whether cytotoxicity of anti-CD20 antibodies could be enhanced by manipulating metabolism.

Results: Intracellular calcium concentration was decreased across our panel of cell lines following a 24-hour treatment with all Type-II anti-CD20 antibodies in our panel. This decrease was not observed following treatment with the Type-I anti-CD20 antibody Rituximab. Treatment with anti-CD20 antibodies resulted in a significant increase in the maximal respiratory capacity of our panel of cell lines; cells were able to produce more ATP in response to oxidative stress. Conversely, we observed a reduction in the respiratory capacity of cells following treatment with the Type-II anti-CD20 antibodies. Next, we show anti-CD20 antibody treatment causes cells to increase maximal mitochondrial respiratory capacity to compensate for reduced basal mitochondrial function. We show that inhibition of OxPhos disables the cells from being able to increase mitochondrial respiratory capacity observed with anti-CD20 antibody treatment alone. When analysing the clonogenic survival of cell lines, we have found that only the cytotoxicity of Type-II anti-CD20 antibodies is enhanced by simultaneously treating cell lines with Metformin.

Summary/Conclusions: Our data show for the first time that when cells are treated with Type-II anti-CD20 antibodies, intracellular calcium is decreased. Intracellular calcium remains unchanged following treatment with Rituximab. Next, we show anti-CD20 antibody treatment causes cells to increase maximal mitochondrial respiratory capacity to compensate for reduced basal mitochondrial function. We show that inhibition of OxPhos disables the cells from being able to increase mitochondrial 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.

E1381

CYCLIN D1 ONCOGENIC OVEREXPRESSION LEADS TO A GLOBAL TRANSCRIPTIONAL DOWNREGULATION IN MALIGNANT LYMPHOID CELLS

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Background: Cyclin D1 is an oncogene frequently overexpressed in human cancers. In hematologic neoplasms, mantle cell lymphoma and multiple myeloma are clear examples of deregulated cyclin D1 expression. It plays a dual function as cell cycle and transcriptional regulator, although the latter is widely unexplored.

Aims: In this study, we investigate the transcriptional role of cyclin D1 in lymphoma and mantle cell lymphoma (MCL). In particular, we compared cyclin D1 oncogenic overexpression in B cells as a model of the first steps in MCL oncogenesis.

Methods: Chromatin immunoprecipitation (ChiP) followed sequencing was performed in four established MCL cell lines. RNA-Sequencing (RNA-Seq) and information from histone ChiP-Seq were correlated with genomic intervals displaying cyclin D1 binding. Transcriptional downregulation was studied through cytomtric 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. Cyclin D1 overexpression was responsible for a global transcriptional down-regulation. 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 CLASSICAL IMMUNE-MEDIANATED MCL 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 stages of HL, while 44.6% - with advanced stages. ABVD or BEACOPP (14/15) were administered as a 1st-line therapy. 78.3% of patients achieved remission (CR/PR), while 8.1% had progression of disease during the therapy. We recorded 14.8% relapses in patients after the 1st line therapy during the follow-up period (median duration – 36 months; range 6-66 months). PD-L1, PD-L2, IDO, TGF-β, IL-13 mRNA expression levels were analyzed in fresh pre-treatment lymph nodes biopsies using qRT-PCR.

Results: Expression of PD-L1 ligands was heterogeneous across the samples and did not depend on histological variant or stage of cHL. Only 12.1% of patients (9/74) were PD-L1 negative and all but one of those cases had a CR and a long-term remission. Patients with PD-L1 overexpression tended to have a higher risk of relapse, comparing to low positive 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=0.006). 85.7% (6/7) and 23.3% (7/30) of relapses were observed during the follow-up period in IDO+ and IDO-patients, respectively (p<0.05). The patients with double negative expression of PD-L1 and IDO were noted to have a favourable outcome of cHL. A 5-year event-free survival (EFS) 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 analyzed with histological variants of relapsing HL, however 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 suggest that tumor microenvironment plays an important role in clinical behavior of cHL. Hence, better understanding of molecular mechanisms of interaction between tumor and immune cells probably can provide us with a novel promising strategy for relapsed/refractory cHL treatment.

E1383
AN IN VIVO TRACEABLE AND MULTIPLYING CRISPR/CAS9 GENOME EDITING SYSTEM
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Background: Gene gain of function and loss of function mutations, oncogene overexpression, gene amplification, chromosome deletion and epigenetic changes, may lead to lymphoma onset. The CRISPR-Cas9 genome editing system has become a feasible tool for exploring the functions of specific genes in different contexts. We want to use this technique to screen for lymphoma suppressor genes.

Aims: Construct an in vivo traceable and multiplying CRISPR-Cas9 gene editing system, which is high efficient for studying in vivo functions of both individual genes or any given chromosome fragment.

Methods: Two retroviral vectors were constructed via molecular clone, one of which contains a locus for tandem U6-sgRNAs and inducible GFP reporter gene and the other contains Cas9 and MS4A1 coding gene. This system’s function of traceable and simultaneously mutate multiple gene efficiencies were validated in vitro. Eμ-myc HSPCs retrovirally transduced with sgp53 and Cas9 were transplanted into sublethally irradiated C57/BL6 mouse.

Results: Co-transduced cells can be tracked by the expression of GFP protein and multiple sgRNA can be efficiently introduced to the GFP-labeled cells for simultaneously mutating multiple genes or deleting a large chromosome fragment. Further we applied this system for both in vitro and in vivo genome editing. As an example, we show that Tp53 mutation accelerated Eμ-Myc driven lymphoma onset in vivo.

Summary/Conclusions: This traceable and multiplying CRISPR/Cas9 system might be useful for various genome editing applications.

E1384
Abstract withdrawn.

E1385
HDAC6 INHIBITION SENSITIZES TUMOR CELLS TO ANTI-CD20 IMMUNOTHERAPY IN VIVO
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Background: Down-regulation of CD20, a molecular target for monoclonal antibodies, constitutes a clinically significant issue leading to decreased efficacy of anti-CD20-based therapeutic regimens. The epigenetic modulation of CD20 coding gene (MS4A1) has been proposed as a mechanism for the reduced therapeutic efficacy of anti-CD20 antibodies and confirmed previously with clinically available non-specific histone deacetylase pan-inhibitors (HDACis). However, the identification of particular HDAC isoforms involved in CD20 regulation seems to be of paramount importance. Since the use of pan-HDACi is associated with substantial side effects, especially difficult to manage in elderly and frail patients, the new specific HDAC6 inhibitors are currently being tested in multiple myeloma and non-Hodgkin lymphoma. They have already been shown to sensitize tumor cells to proteasome inhibitors and novel kinase inhibitors e.g. Ibrutinib and demonstrated promising results in in vitro studies in chronic lymphocytic leukemia (CLL).

Aims: HDAC6 has been known for its regulatory role in protein degradation. We have previously reported that inhibition of proteasome activity can effectively increase CD20 levels in tumor cells. In our study we tested the hypothesis that selective HDAC6 inhibition sensitizes tumor cells to immunotherapy with anti-CD20 mAbs. The aim of the study was to test the hypothesis that selective HDAC6 inhibition sensitizes tumor cells to immunotherapy with anti-CD20 mAbs.

Methods: We assessed the influence of HDAC6 inhibition in a panel of different subtypes of human lymphoma cell lines (Burkitt, DLBCL: both EBV+ and EBV- cells) on CD20 expression using flow cytometry and Western blotting. We confirmed our observations in primary samples from the patients with CLL, 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 nicotinostat) 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 rituximab i.p. We used both the pharmacological (i.p. administration of nicotinostat) 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 direct cytotoxicity of obinutuzumab. 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-CD20 mAbs. This strategy seems to be highly promising in CLL patients, often expressing very low CD20 level and do not fully benefiting from immunotherapy.

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E1386
NKP46 EXPRESSION IS A DIAGNOSTIC AND PROGNOSTIC BIOMARKER IN PRIMARY GASTROINTESTINAL T-CELL LYMPHOPROLIFERATIONS. A CELAC NETWORK STUDY
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Background: NKG2D ligands, such as NKL3 and Nkp46, have been identified as markers of normal cytotoxic T lymphocytes with NKG2D receptors. While NKL3 expression is generally low, Nkp46 expression is elevated in a variety of malignancies. There are only a few studies investigating NKp46 expression in gastrointestinal T-cell lymphoproliferations (GTCLs), including lymphomatoid papulosis (LP) and enteropathy-associated T-cell lymphoma (EATL).

Aims: To investigate the expression of Nkp46 in different subtypes of gastrointestinal T-cell lymphoproliferations (GTCLs), including lymphomatoid papulosis (LP) and enteropathy-associated T-cell lymphoma (EATL).

Methods: We performed immunohistochemical analysis of Nkp46 expression in a cohort of 102 patients with gastrointestinal T-cell lymphoproliferations (GTCLs), including lymphomatoid papulosis (LP) and enteropathy-associated T-cell lymphoma (EATL).

Results: Nkp46 expression was detected in 20% of patients with LP and 30% of patients with EATL. The expression of Nkp46 was significantly higher in patients with EATL compared to patients with LP. The expression of Nkp46 was also significantly higher in patients with EATL than in patients with other subtypes of gastrointestinal T-cell lymphoproliferations (GTCLs).

Summary/Conclusions: Our results suggest that Nkp46 expression is a diagnostic and prognostic biomarker in primary gastrointestinal T-cell lymphoproliferations. The expression of Nkp46 is significantly higher in patients with EATL compared to patients with LP and other subtypes of gastrointestinal T-cell lymphoproliferations (GTCLs).
INDUCTION OF APOPTOSIS

Background: Primary gastrointestinal (GI) T-cell lymphoproliferations (T-CL) are heterogeneous entities, which diagnoses are difficult to perform. T-CL include aggressive lymphoma such as enteropathy-associated T-cell lymphoma (EATL) as well as indolent monoclonal lymphoproliferations. Refractory coeliac disease type II (RCDII) is one of the indolent clonal T-GL that complicates coeliac disease (CD) and may evolve toward an overt EATL. The differential diagnosis of RCDII from CD and RCDI is difficult and essentially based on negative expression of sCD3 and CD8 and the presence of a clonal TCR rearrangement. Lymphocytes from RCDII are dependent for survival on IL-15, which reprograms T lymphocytes towards a cytotoxic NK phenotype.

Aims: We thus studied the expression of NKp46 on a representative panel of GI T-CL to assess its diagnosis and prognosis value.

Methods: Using formalin-fixed paraffin-embedded tissue biopsies, we assessed NKp46 expression by immunohistochemistry (IHC) and investigated its clinical and biologic significance on 177 intestinal, 11 lymph node and 5 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_MUTL, 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 on indolent T-LPD (n=0/15). The NKp46 expression was also associated with a poor prognosis in GI T-cell lymphoma patients (OS-5years 50.5% vs 5.4%, P=0.0011) (Figure 1B).

Summary/Conclusions: The NKp46 expression in more than 25 IEL per 100 EC by IHC analysis can easily identify RCDII from CD and RCDI. Furthermore, the NKp46 expression is associated with aggressive forms of GI T-cell lymphoma. Finally, the NKp46 expression was strongly associated with shortened survival. Thus NKp46 provides a new biomarker for both diagnosis and prognosis in GI T-CL.
hours and produced 8.7 ~ 9.3 X 10^3 ng/ml of IgM). PCs isolated from BCWM.1 increased to 130% and produced 2.5 ~ 2.8 X 10^4 ng/ml of IgM. LPLs from both cells as well as in SNK-6 cells. When comparing with those transfected with IL-2Rα-harboring lentiviral vectors, and the smaller amounts of IgM than PCs (3.5 ~ 5.0 X 10^3 ng/ml in MWCL-1 and 0.3 in BCWM.1 at 72 hours), gave rise to the more differentiated PCs (7.5 ~ 9.0% in BCWM-1 and 1.2 ~ 1.4% PCs in BCWM.1), and secreted increased to 130% and produced 2.5 ~ 2.8 X 10^4 ng/ml of IgM. LPLs from both cells as well as in SNK-6 cells. When comparing with those transfected with IL-2Rα-harboring lentiviral vectors, and the smaller amounts of IgM than PCs (3.5 ~ 5.0 X 10^3 ng/ml in MWCL-1 and 0.3 in BCWM.1 at 72 hours), gave rise to the more differentiated PCs (7.5 ~ 9.0% in BCWM-1 and 1.2 ~ 1.4% PCs in BCWM.1), and secreted significantly higher in SNK-6 cells than in NK-92 cells transfected with LMP-1-harboring lentiviral vectors, and the in the downstream pathways of LMP1 signaling were measured in NK-92 cells and explored the role of IL-2Rα in lymphomagenesis and 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 UREGULATION 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 state of T-cell exhaustion; and ultimately provokes tolerance towards cancer cells. PD1 is expressed on Hodgkin lymphoma (HL) and B-cell non-HLTL-Cells. 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 inhibitors. The TME, including those of Tg-l cells and NK cells, can modulate cytotoxicity in experimental models. Preclinical findings indicate that combination of IMIDs with immune checkpoint inhibitors may promote therapeutic synergy and long-term antitumor immunity to improve clinical outcome.

**Aims:** 1) To better characterize the PD1, PDL1 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 PD1 axis 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 gene expression 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 Celgene) 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 PDL1 was similarly expressed, while PDL2 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 increased PD1 expression and very low levels of ligands were detected in both CD4+ and CD8+ cells. Co-culture of lymphoma with T-cells resulted in co-stimulatory effect on T-cell proliferation and the consistent formation of T/B-cell clusters. Higher numbers of CD19+CD52+PD-L1+ 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 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 PD1L1 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.

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 cell lines were examined with MTT assay and flow cytometric analysis. Expression of BCL6 and its target genes was examined with quantitative RT-PCR and western immunoblotting. The therapeutic efficacy of ATO treatment was also examined in a DLBCL (OCI-Ly7) xenograft mouse model.

Results: OCI-Ly1, OCI-Ly7 and SU-DHL-6 were highly sensitive to inhibitory activity of BCL6 inhibitor and were designated as BCL6-dependent. Treatment of DLBCL cells with ATO led to a decrease in BCL6 protein level and an upregulation of downstream targets of BCL6, including PRDM1, CD44 and CD69. The effect of ATO on BCL6 protein were abrogated by treatment with proteasome inhibitors and therefore, ATO led to BCL6 for degradation through the ubiquitin-proteasome pathway. Interestingly, ATO also inhibited cell proliferation and induced apoptotic cell death of BCL6-dependent DLBCL cell lines, analogous to the effect of BCL6 inhibitor on these cells. In addition, there was a synergistic inhibitory and cytotoxic activity between ATO and cisplatin. Finally, ATO treatment suppressed the growth of DLBCL in a xenograft mouse model.

Summary/Conclusions: ATO targets BCL6 for proteasomal degradation and inhibits the proliferation and growth of BCL6-dependent DLBCL.

Arsenic trioxide targets BCL6 for degradation and inhibits the proliferation of BCL6-dependent diffuse large B-cell lymphoma

E1391

IDENTIFICATION AND DIAGNOSTIC APPLICATION OF GENOMIC NPM-ALK FUSION TRANSCRIPTS IN ANAPLASTIC LARGE CELL LYMPHOMAS

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Background: ALK positive anaplastic large-cell lymphomas (ALCL) account for 10-15% of pediatric Non-Hodgkin lymphomas. Most of these patients carry the chromosomal translocation t(2;5)(p23;q35) leading to the fusion of the NPM and ALK genes. The genomic NPM-ALK fusion transcripts is a well-established tool for diagnostic purposes and risk stratification during the course of treatment.

Aims: Establishment of a PCR based assay to identify patient-specific genomic NPM-ALK fusion sequences for a DNA based monitoring of minimal residual disease in ALCL patients. Compared to RNA based methods the quantification of DNA is independent of the gene expression. Additionally, due to the higher stability of DNA, cell-free circulating tumor DNA (ctDNA) should be detectable in the patient’s plasma and may represent a tumor marker for ‘liquid biopsies’ in ALCL.

Methods: Using a specifically designed multiplex long-range PCR assay, genomic NPM-ALK fusion sequences were identified in 45 ALCL patients. The genomic NPM-ALK breakpoints were analyzed concerning fine structure and breakpoint distribution pattern. Furthermore, the patient-specific genomic NPM-ALK fusion sequences were evaluated for their use as biomarkers in selected cases. For this purpose patient’s blood and plasma samples were quantified using a high sensitive digital droplet PCR assay.

Results: In more than 60% of cases the identified breakpoint was localized within repeat regions. The genomic breakpoints within the breakpoint cluster regions of the fusion genes were randomly distributed. Most of the NPM-ALK fusion sequences were characterized by the occurrence of small insertions or deletions indicating the involvement of the non-homologous end-joining (NHEJ) repair system for chromosomal translocation initiation. Using a DNA based quantification assay in a subset of patients, the genomic NPM-ALK fusion sequences were detectable in circulating tumor cells in patient’s blood samples as well as cell-free tumor DNA in plasma samples.

Summary/Conclusions: The established multiplex long-range PCR assay is a useful diagnostic tool for the identification of genomic NPM-ALK fusion sequences. This individual tumor maker is independent of gene expression and can be used for therapy response monitoring and relapse detection.

E1392

ARSENIC TRIOXIDE TARGETS BCL6 FOR DEGRADATION AND INHIBITS THE PROLIFERATION OF BCL6-DEPENDENT DIFFUSE LARGE B-CELL LYMPHOMA

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Background: B-cell lymphoma 6 (BCL6) is a transcription repressor and is constitutively expressed in most cases of diffuse large B-cell lymphoma (DLBCL). BCL6 expression is important for the survival of transformed B-cells and promotes the formation of active B-cells. The quantification of BCL6 expression of PD1 in CD8+ and CD4+ cells and may contribute to reactivate PD1 signaling under treatment. The PD1 pathway may be potentially targeted to overcome both the intrinsic and LEN-induced exhaustion phenotype.

Aims: To investigate if ATO targets BCL6 and inhibits the proliferation and growth of BCL6-dependent DLBCL.

Methods: BCL6-dependency of a panel of DLBCL cell lines (i.e. OCI-Ly1, OCI-Ly7, SU-DHL-6, OCI-Ly18 and Pfeiffer) was determined based on their sensitivity to proliferation inhibitory activity of the BCL6 inhibitor 79-6 (Calbiochem). The effects of ATO and cisplatin as single agent or in combination on cell viability and apoptosis of DLBCL cell lines were examined with MTT assay and flow cytometric analysis. Expression of BCL6 and its target genes was examined with quantitative RT-PCR and western immunoblotting. The therapeutic efficacy of ATO treatment was also examined in a DLBCL (OCI-Ly7) xenograft mouse model.

Results: OCI-Ly1, OCI-Ly7 and SU-DHL-6 were highly sensitive to inhibitory activity of BCL6 inhibitor and were designated as BCL6-dependent. Treatment of DLBCL cells with ATO led to a decrease in BCL6 protein level and an upregulation of downstream targets of BCL6, including PRDM1, CD44 and CD69. The effect of ATO on BCL6 protein were abrogated by treatment with proteasome inhibitors and therefore, ATO led to BCL6 for degradation through the ubiquitin-proteasome pathway. Interestingly, ATO also inhibited cell proliferation and induced apoptotic cell death of BCL6-dependent DLBCL cell lines, analogous to the effect of BCL6 inhibitor on these cells. In addition, there was a synergistic inhibitory and cytotoxic activity between ATO and cisplatin. Finally, ATO treatment suppressed the growth of DLBCL in a xenograft mouse model.

Summary/Conclusions: ATO targets BCL6 for proteasomal degradation and inhibits the proliferation and growth of BCL6-dependent DLBCL.

E1393

PROTEOMIC PHOSPHOSITE ANALYSIS IDENTIFIED CRUCIAL NIPA SERINE RESIDUES FOR NPM-ALK-MEDIATED TRANSFORMATION

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Background: Anaplastic large-cell lymphoma(ALCL) is an aggressive non-Hodgkin lymphoma that occurs mainly in children and younger adults. Patients typically show an advanced stage disease as well as an aggressive disease pattern with 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 autocrine NPM-ALK expression. Nuclear interaction partner of ALK (NIPA) was first identified as a new interaction partner of the oncogene NPM-ALK in a yeast-2-hybrid screen which defines an E3-SCF ligase and is physiologically involved in cell cycle regulation at the transition from G2 phase to mitosis. It has already been shown in preliminary studies that co-expression of NIPA with the oncogenic tyrosine kinase NPM-ALK results in the constitutive phosphorylation of NIPA (Illert et al., 2012a). Until now, the specific signal transduction pathway, the crucial phosphorylation sites in NIPA as well as the functional effect of the pathological NIPA phosphorylation in NPM-ALK-induced lymphomagenesis still remain unclear. Molecular insights into the molecular mechanism involved in the oncogenic NPM-ALK may help to identify new druggable targets for therapeutic implications.

Aims: In the present study, we investigated the molecular mechanisms as well as the functional impact of the NPM-ALK-induced NIPA phosphorylation.

Methods: For this purpose, biochemical methods with ALCL cells were used to examine functional effects of constitutive and NIPA 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: MITT proliferation- and Softagar-Assays were performed after cell infection of BaF3 and primary NIPA-deficient MEF cells with NPM-ALK and the respective phospho-deficient NIPA to reveal transformation and growth ability.

Results: It has already been shown, that cell cycle dependent NIPA phosphorylation at critical serine residues 354, 359 and 359 leads to dissociation of the inactive NIPA and the respective phospho-deficient NIPA to reveal transformation and growth ability.

Summary/Conclusions: Taken together, we identified five phosphorylation sites in NIPA to be highly upregulated upon NPM-ALK expression. However,
E1394
APPLICATION OF CELL-OF-ORIGIN SUBTYPES DETERMINED BY DIGITAL GENE EXPRESSION IN HIV-RELATED DIFFUSE LARGE B CELL LYMPHOMAS

Methods: A series of HIV-related DLBCL using the Lymph2Cx assay and to compare the cell-of-origin (COO) given by Hans algorithm and Lymph2Cx assay.

Results: The median follow-up of living patients was 8.5 years. IHC studies showed that 35.8% of the cases expressed CD10, 61.5% expressed BCL6, 55.8% expressed MUM1, and according to Hans algorithm 56.6% had a non-GC phenotype. CD30 was expressed in 15.4% of the cases and EBER was found in 21.2%. The expression of MYC was detected in 32.7% of the cases and BCL2 in 44%, and 18% were dual expressers. Rearrangements involving MYC, BCL2 and BCL6 were detected in 26%, 8% and 28%, respectively. The Lymph2Cx assay assigned a COO to all 55 studied cases. 63.6% were GCB subtype, 20% were ABC subtype, and 16.4% were unclassified. The only clinical feature significantly associated with a defined COO subtype was B-symptoms (ABC=81.8% vs GCB=28.6%, P=0.003) and HIV-load tended to be more frequently observed in ABC (90%) than in GCB (58.1%, P=0.068). Regarding IHC, in the follow-up results, MYC rearrangements were only detected in GCB cases and expression of CD10 and BCL6 tended to be associated with GCB (Table 1). Hans algorithm and Lymph2Cx assay differently assigned COO subtypes (κ=0.288, P=0.029) showing that 44.1% of the GCB cases had a non-GC phenotype according to Hans. Only patients treated with RCHOP were considered in survival analyses (N=47). COO subtypes had neither impact on OS nor PFS, independently of being determined with Hans or Lymph2Cx assay. Features associated with shorter OS and PFS were history of AIDS-defining illnesses, HCV-infection and dual MYC and BCL2 expression. Extranodal disease and increased MYC or BCL2 expression were also bad prognostic factors for PFS.

Summary/Conclusions: In HIV-related lymphomas, COO subtypes were discordantly assigned with Hans and Lymph2Cx assay and COO subtypes showed no impact on outcomes, independently of the method applied.

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E1395
CXC4R 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 tumourigenesis 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 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 and to analyse the effects of CXCR4 antagonists on cell growth and migration of aggressive lymphoma cells in vitro.

Results: By correlating CXCL12 expression levels of infiltrated bone marrow biopsies, we observed a very strong significant negative correlation between CXCL12 expression and the percentage of infiltration levels (Spearman-Rho=0.764; p=0.001). Furthermore, remission in bone marrow after standard immunochemotherapy was associated with a reduction of CXCR4 expression (p=0.075). The cell growth of BL2 and RI-1 cell lines -exhibiting strong and moderate CXCR4 expression- was significantly inhibited by AMD070 and WK1 (p<0.01). As the cell line case of U2932 -exhibiting low CXCR4 expression- was just affected by WK1. AMD3100 did not show any effects on the lymphoma cell growth. The transmigration index to evaluate the chemotactic ability of lymphoma cells was reduced by AMD070 and WK1 treatment, however, the inhibitory effects of WK1 were lower compared to AMD070.

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.
EPSTEIN-BARR VIRUS LOAD IN PLASMA IS AN EARLY BIOMARKER OF HIV-RELATED LYMPHOMA

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Background: Epstein Barr virus (EBV) has been detected in the tumor cells of some non-Hodgkin lymphomas (NHL) and Hodgkin lymphomas (HL) and detectable EBV loads have been found in the plasma of immunocompetent patients with HL. In HIV-related lymphomas the importance of EBV load as potential biomarkers has been scarcely studied.

Aims: We aimed to evaluate the usefulness of EBV load in plasma as lymphoma biomarker in HIV-infected patients.

Methods: One hundred and fifteen patients with NHL (HIV-infected =57 and HIV-uninfected=34) and HL (HIV-infected= 16 and HIV-uninfected= 8) were studied. EBV loads were determined in plasma by means of a commercial real-time PCR technique (EBV PCR kit, Qiagen GmbH, Hilden, Germany) at lymphoma diagnosis and at complete response (CR) in 1 group of HIV-infected patients also at one year before diagnosis (N=11) and at complete response (CR) (N=34). EBER 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 qualitative and quantitative variables, respectively. Survival analyses were performed using the Kaplan-Meier method. P-values of less than 0.05 were considered statistically significant.

Results: At diagnosis, EBV loads were detectable in more HIV-infected patients than HIV-uninfected (48% vs 14%, P<0.002) and in more HL cases than NHL (70% vs 26.3%, P<0.006). In HIV-infected patients, detectable EBV load was associated with EBER expression, 66.6% of the patients with detectable EBV loads had EBER-positive tumors and 92% of the patients with undetectable EBV loads had EBER-negative tumors (P=0.003). All the remaining clinical and biological features were not associated with detectable EBV load in plasma. In HIV-uninfected patients, associations between EBV load and EBER expression (P=0.006) and EBV load and HVB infection (P=0.017) were observed. From 16 out of 34 (47%) HIV-infected patients with detectable EBV loads at lymphoma diagnosis, 15 had undetectable EBV loads at CR (P=0.001) (Figure 1). The exception was one patient with HL whose EBV load substantially decreased at CR but was still detectable. Moreover, 4 out of 7 HIV-infected patients with detectable EBV loads at diagnosis had detectable loads one year before diagnosis, and no patient with negative EBV loads at diagnosis had detectable loads before it, pointing EBV load can be used as an early biomarker of lymphoma. EBV loads at diagnosis had neither impact on overall survival nor progression-free survival

Summary/Conclusions: EBV-load in plasma can be used as early biomarker of lymphoma in HIV-infected patients since EBV-loads can be detected up to 1 year before lymphoma diagnosis and are virtually undetectable at lymphoma CR.

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CLONOTYPE AND MUTATIONAL PATTERN IN TCRGD LARGE GRANULAR LYMPHOCYTE LEUKAEMIA

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Background: T-cell large granulocytic lymphocytic leukemia (T-LGLL) is a rare heterogeneous T-cell neoplasia whose leukemic cells usually express the CD3 T-cell receptor (TCR); only a small subset of cases expresses the γδ TCR denoting the TCRγδ LGLL. Currently, among the different LGL diseases, TCRγδ LGLL remains less studied and several clinical and laboratory data already described in TCRαβ-LGLL have not yet been explored in TCRγδ-LGLL.

Aims: The aims of this work were 1) to characterize TCRγδ-LGLL defining STAT mutational pattern and CDR3 repertoire diversity/clonal composition (clonotype) and 2) to evaluate correlations among LGL phenotype, mutations, TCR rearrangement and clinical presentations.

Methods: In this work 11 patients affected by TCRγδ-LGLL were included. Sanger sequencing was used for mutational analysis on hot-spot regions in the two genes more frequently mutated in LGL disorders, STAT3 and STAT5b. Immunophenotype of LGL clone was defined by flow cytometry analysis. CDR3 repertoire and frequency distribution of TCR gamma gene rearrangements were analyzed.

Results: Our results showed that TCRγδ LGLL had a high incidence of STAT mutations, 9 out of 11 patients carrying STAT3 or STAT5b mutations in a mutually exclusive pattern. At variance from CD8+ TCRαβ LGLL and CD4+ TCRδ LGLL, first being mostly characterized by STAT5b mutations and the latter by STAT5b, TCRγδ LGLL patients were characterized by both the mutations. Thus, TCRγδ LGLL showed features shared by CD8 and CD4 TCRαβ-LGLL. Consistently, TCRδ LGLL showed the same correlation between immunophenotype and kind of mutation observed in TCRαβ-LGLL: γδLGLL patients with CDδ1+CD56- LGL immunophenotype were characterized by STAT3 mutations (as in CD8+ T-LGLL), while γδLGLL patients with CDδ6+ LGL immunophenotype by STAT5b mutations (as in CD4+ T-LGLL). Moreover, we observed that patients with γδLGLL 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, Vg3-Jg1/2, Vg9-JgP and Vg8-Jg1/2 were the combination usages most frequently detected. Concerning the clonotype repertoire, CDR3 sequences of the immunodominant clones were 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 TCRγδ LGLL can be considered as the interplay of the two types of TCRαβ-LGLL, sharing some CDR3 and TCRγδ LGLL mutational features. As already described in TCRαβ-LGLL, also in γδLGLL, the first being mostly characterized by STAT5b mutations and the latter by STAT3 mutations, CD8 and CD4 TCRαβ-LGLL. Thus, TCRγδ LGLL patients were characterized by both the mutations. STAT5b mutations seems to be more frequently linked to monoclonal nature of the LGL lymphoproliferation. Rather, the marker Vδ1 appears to be correlated to symptomatic disease.

INCREASED EXPRESSION OF IRF8 IN TUMOR CELLS INHIBITS THE GENERATION OF TH17 CELLS AND PREDICTS UNFAVORABLE SURVIVAL OF DIFFUSE LARGE B CELL LYMPHOMA PATIENTS

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Background: The immunological pathogenesis of diffuse large B cell lymphoma (DLBCL) remains elusive. Searching for new prognostic markers of DLBCL is a crucial focal point for clinical scientists.

Aims: The aim of the present study was to examine the prognostic value of interferon regulatory factor 8 (IRF8) expression and its effect on the development of Th17 cells in the tumor microenvironment of DLBCL patients.

Methods: Flow cytometry, immunohistochemistry, and quantitative real-time PCR were used to detect the distribution of Th17 cells and related cytokines and IRF8 in tumor tissues from DLBCL patients. Two DLBCL cell lines (OCI-
LY10 and OCI-LY1 with IRF8 knockdown or overexpression and two human B lymphoblast cell lines were co-cultured with peripheral blood mononuclear cells (PBMCs) in vitro to determine the effect of IRF8 on the generation of Th17 cells. Quantitative real-time PCR and Western blotting were used to investigate the involvement of retinoic acid receptor-related orphan receptor gamma t (RORγt) in the effect of IRF8 on Th17 cell generation. The survival of 67 DLBCL patients was determined using the Kaplan-Meier method and log-rank analysis.

**Results:** The percentage of Th17 cells was lower in DLBCL tumor tissues than in PBMCs and corresponding adjacent benign tissues. Relative expression of interleukin (IL)-17A was lower, whereas that of interferon (IFN)-γ was higher in tumor tissues than in benign tissues. Co-culture with DLBCL cell lines inhibited the generation of Th17 cells in vitro. IRF8 upregulation was detected in DLBCL tumor tissues, and it was associated with decreased DLBCL patient survival. Investigation of the underlying mechanism suggested that IRF8 upregulation inhibited Th17 cell generation by suppressing the effect of RORγt on CD4+ T cells.

**Summary/Conclusions:** Our findings suggest that IRF8 expression in the tumor microenvironment inhibited the generation of Th17 cells through its antagonistic effect on RORγt in the DLBCL tumor microenvironment, suggesting that it could be a prognostic factor for DLBCL.

**E1399**

**GENOMIC PROFILING OF BCL2 AND MYC DOUBLE EXPRESSION 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 expression (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/IgH (Zytovision) double-fusion probe. Infinium HD whole-genome genotyping assay with the HumanCytoSNP FFPE-12 BeadChip Immuna, San Diego, CA, USA was performed for genomic analysis of the aberrations.

**Results:** A cohort of 91 primary DLBCL patients diagnosed between 2004 and 2012 was selected for the study. Immunohistochemical evaluation was informative in 87 (95.6%) cases. Genomic analysis, 61 cases were informative for MYC, 56 cases for Bcl6, and 65 cases for Bcl2. 7 cases (11.4%) were positive for MYC translocation, 14 (25%) for Bcl6, and only 3 (4.6%) were positive for Bcl2. No cases of MYC BCL2 and double positive DLBCL were identified. Genomic DNA from FFPE tumor tissue for SNP-A was available in 61 cases, SNP-A analysis was performed in total 329 patients. Abnormalities not corresponding to known copy number polymorphisms (89% of all the patients, 59/66). These comprised 164 (50%) hemizygous and 2 (1%) homozygous deletions, 106 (32%) gains, 41 (12%) trisomies and 16 (5%) monosomies. The most common aberrations were 1p deletion, 1q gain, 6q deletions with trisomy 12, and 17q (karyotype >3 aberrations) was detected in 37/66 (56%) patients. Both DE and non-DE DLBCL groups had equal rate of aberrations per case (~5 aberr./case) and shared the most common aberrations – 1p deletion and 1q gain. In contrast, 11q deletion was more common in DE, while 6q and 17q deletions were more prevalent in the non-DE group.

**Summary/Conclusions:** SNP-A analysis highlights the genomic differences between the DE and non-DE DLBCL. Our finding of MYC positive (translocations and/or gains) association with low complexity karyotype status may suggest MYC to be an early initiating genetic event.

**E1400**

**ARQ 531, A REVERSIBLE BTK INHIBITOR, DEMONSTRATES POTENT ANTI-TUMOR ACTIVITY IN ABC-DLBCL AND GCB-DLBCL**

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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) and 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 additional activity of Src, Trk family members, as well as additional in vivo response in ABC and GCB patient groups.

**Methods:** Biochemical inhibition and kinase profiling were assessed using recombinant proteins. The ARQ 531 binding kinetics on BTK were determined by Surface Plasmon Resonance assay. Anti-proliferative activity of ARQ 531 was tested in a MTS-based assay against a panel of hematological malignant cell lines. Pathway inhibition assessments, in vivo efficacy and in vivo target inhibition were performed in TMD8 (ABC-DLBCL) and SUDHL4 (GCB-DLBCL) cell lines and xenografts. ADME and pharmacokinetic properties of ARQ 531 were also evaluated in rats, dogs and monkeys.

**Results:** ARQ 531 potently inhibited BTK (IC50=0.85 nm) and displayed long half-life (56 min). ARQ 531 exhibited strong anti-proliferative activity in TMD8 (GL50=1.13 μM) and SUDHL4 (GL50=0.2 μM) cell lines. Ibrutinib, while potent on TMD8 cells (GL50=0.002 μM), had a GL50 of 1.1 μM in SUDHL4, a concentration not reached in human blood, consistent with published studies. Pathway analysis in TMD8 and SUDHL4 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 92% after 14 days of dosing, with no re-growth observed for 17 days post dose interruption. In the ibrutinib-resistant SUDHL4 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 selective kinase selectivity can be used to target constitutive BCR signaling in DLBCL primarily resistant to ibrutinib, as demonstrated by the excellent efficacy in both ABC and GCB DLBCL xenograft models. These data support the clinical investigation of ARQ 531 in patients with hematological malignancies, expected to begin in mid-2017.
vival (OS). The efficacy of R-CHOP was evaluated according to Cheson criteria by performing standard hematochemical and instrumental (TC and FDCG-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 Affymetrix array. To date, 21 SNPs from 19 candidate genes (ABC1B, ABC1C, ABC2C, ABC2G, CDK4, CDK6, DOR1, FHC1, FGF2, FGFRA, GATA2, GATA3, GATA4, GSTP1, IL1H, NCF4, NOQ1, NOQ2, RAC2, TNF, TOP2A, TP53, TUB1) 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 outcomes. Case-control analyses were performed using Risk genotypes (RR) and one or two minor alleles (RR/RR, RR/RQ) as a predictor of PFS (p=0.045). Pts with HR or RR genotypes showed shorter PFS than pts with HT genotype (HR: 2.437, 95% CI 1.00-5.823). No statistically significant correlation was found between SNPs and OS.

**Summary/Conclusions:** Our preliminary data obtained in a limited number of pts, show a association between a SNP of the low affinity FGFR2A gene involved in the activity of rituximab and PFS. Further insights will derive from the completion of pts 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 (11;14) resulting in a constitutive cyclin D1 overexpression. The cyclin D1-CDK4/6 complex inactivates Rb through phosphorylation, leading to G1/Phase transition. Therefore, inhibition of CDK4/6 is an efficient and rational approach to overcome cell cycle dysregulation in MCL.

**Aims:** We evaluated the efficacy 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, Mav-1, Mino) and primary MCL cells were exposed to abemaciclib alone and combined with AraC or Ibrutinib. Pts 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 for 31,25 µM abemaciclib and 2 µM Ibrutinib. Whereas the simultaneous application of abemaciclib and ibrutinib had a protective effect against AraC treatment in all sensitive cell lines of MCL. Sequential administration of abemaciclib followed by AraC showed strong synergy in Mino cells (CI=0,33 µM AraC). Western blot analysis of abemaciclib and ibrutinib had synergistic or additive effects in sensitive cell lines (Cis: JeKo-1=0,24; Mav-1=0,19; Mino-0,03 for 31,25 µM abe and 2,5 µM ibru), whereas the simultaneous administration of both showed additive effects at most (Cis: JeKo-1=0,24; Mav-1=0,1; Mino-0,09 for 31,25 µM abe and 2,5 µM ibru). In primary MCL cells abemaciclib had no impact on cell death or sensitization since no cell proliferation was observable and cells where resting in G1-phase.

**Summary/Conclusions:** The novel CDK4/6 inhibitor abemaciclib causes reversible G1 cell cycle arrest without loss of viability at low nanomolar doses. Rationale drug combinations exploiting the sequential effect may achieve major benefits. Pretreatment with abemaciclib might sensitize cells to ibrutinib, resulting in synergistic drug effects. In contrast, simultaneous application of abemaciclib protects cells from AraC treatment whereas Abemaciclib-induced S-phase synchronization sensitizes MCL cell lines to AraC. Further analysis is needed to explore the interaction with other targeted approaches (inhibitors of the B-cell receptor pathway) to better understand the underlying molecular mechanisms.
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 immunohistochemical subtype of diffuse large B cell lymphoma (DLBCL). This CD5+ DLBCLs, either de novo lesions or transformed from preceding low grade lymphomas, can only be identified in LN for the majority of patients with aggressive behavior. The incidence of CD5+ DLBCL was variably reported from 5-22% of all DLBCLs in western countries and Japan, however, no exact data available in Koreans.

Aims: This study aimed to investigate clinicopathologic features of CD5+ DLBCLs.

Methods: A total of 350 cases of DLBCL were reviewed 4 university hospitals from 2004 to 2012. Review of the histologic features along with immunohistochemical study for BCL1, BCL2, BCL6, CD5, CD10, CD23, DM, 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). Richterian transformation was suspicious in 5/30 and EBV was negative in all. Most cases (22/30) belong to non-GCB subtype by Hans classifier. Rearrangement of MYC was found in 2 cases and amplification was found in one. Compared with DLBCL-NOS, CD5+ cases revealed significantly higher expression of MYC, BCL6, IRF4/MUM1 and Ki67 (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 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 particularly aggressive subtype.

Reactive B-lineage lymphoid proliferations (RBLP) in the blood and bone marrow may raise a differential diagnosis of lymphoma.

Aim: 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 pathological reports for samples referred to the Departments of Molecular Medicine and Haematology and Anatomical Pathology 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 definitive evidence of monoclonality.

Results: During this period, 38 patients were diagnosed with florid RBLP with up to 70-80% of cells in blood or bone marrow comprising reactive B cells (including mature B, plasmablasts and plasma cells). All patients tested were HIV positive, with a median age of 28 years (range 6 months-79 years). There was a bimodal age pattern with a peak in children <1 year of age (34% of patients) and a second peak in an age group where the incidence of lymphoma is virtually absent in children under a year of age. Common clinical presentations included cytopenias (85%); infection (70%) (commonly Cytomegalovirus (35%), TB (30%) and bacterial septicaemia (22%)); hepatosplenomegaly (42%); and lymphadenopathy (36%). Patients showed increases in serum total protein levels (reflecting 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).

Table 1. Comparative data: HIV associated lymphoma and HIV associated RBLP.

Summary: In the setting of HIV, reactive conditions may mimic lymphoma and vigilance is needed in the confirmation of monoclonality. Patients with RBLP presented at a younger age when compared to their counterparts with lymphoma. They had extremely high VL with higher CD4 counts, suggesting this may be a feature of early HIV disease and the possibility of a seroconversion type illness should be considered.

MICROVESSEL 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 heterogeneous 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 in patients with diffuse large B-cell (DLBCL) lymphomas.

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 lymphoproliferative disorders with a DLBCL morphology, Primary Mediastinal large B cell lymphoma, Mantle cell lymphoma and Hodgkin lymphoma. Immunohistochemistry was performed as part of the routine workup and CD30 was considered positive at ≥30% staining of neoplastic cells.

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Microvessel quantification was performed by immunohistochemical staining, using monoclonal antibodies against platelets/endothelial cell adhesion molecule-CD31. A total of 82 cases of de novo DBLCL treated with R-CHOP were included in the training set for further analysis. There were 45 men and 37 women, with a median age of 57 years (range, 16–84); 35 patients (43%) presented with B symptoms, and 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.

Methods: 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+ DBLCL 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).

Figure 1. Summary/Conclusions: CD30 is expressed in approximately 29% of all DBLCL and defines a novel subgroup of diffuse large B-cell lymphoma with a more favorable prognosis. Microvessel density expression is lower in CD30 positive DBLCL. 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 RESPONSIBILITIES: HIGH-THROUGHPUT IMMUNOGENETIC EVIDENCE

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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 Istituto Lione Linfomi (FIL) - FIL MRD network - started a collaborative effort to harmonize and standardize their methodologies, performing QC (Quality Control) rounds twice a year for follicular lymphoma (FL) and mantle cell lymphoma (MCL) MRD assessment.

Aims: We evaluated the molecular results of bone marrow (BM) samples analysis performed during the QC rounds, to determine how borderline samples (i.e. those with a low MRD level) challenge the inter-lab reproducibility and data interpretation.

Methods: Between February 2010 and November 2016, in the context of 14 QC rounds, the FIL MRD Network labs received 188 BM (94 FL and 94 MCL) samples; 167 were analyzed by both nested polymerase chain reaction (PCR) and real-time quantitative PCR (RQ-PCR). BCL2/IGH oligoclonal rearrangements were analyzed by nested PCR (Gribben, 1993) and by RQ-PCR (Ladetto, 2000). Clonality assessment was performed using an IGHV multiplex consensus PCR (Ladetto, 2003) and by RQ-PCR (Ladetto, 2000; Donoval, 2000). All analyses were conducted and interpreted according to the “EuroMRD Consortium” guidelines (van der Velden, 2007).

Results: The sensitivity and the accuracy of each molecular analysis was tested, reaching a uniform sensitivity of 10−5 and a quantitative range for RQ-PCR of 10−6 to 10−4. Ninety-thousand cycles were carried out as described (Ladetto, 2000; Donoval, 2000). All analyses were conducted and interpreted according to the “EuroMRD Consortium” guidelines (van der Velden, 2007).

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samples analyzed by both methods. 83% (139/167) of these were classified as +/+ or -/- by all the FIL labs. The remaining 28/167 (17%) were the samples that showed discordant results in the inter-lab assessments: while in 17 cases the "borderline status" was defined alternatively by only one method, 11 resulted brd samples by both techniques (11/167, 6.6%) (Fig.). Given that the 167 samples were tested in three replicates across the 4 labs, a total of 12 replicates/sample were analyzed: 31 brd samples were thus identified, 13 of which brd by both approaches. Of 156 evaluations performed on the 13 brd, 69/156 (44%) resulted PCR-positive and 87/156 (56%) PCR-negative, 58/156 (37%) were RQ-PNQ and 98/156 (63%) RQ-negative.

**Figure 1.**

**Summary/Conclusions:** Despite the high inter-lab reproducibility in the MRD analysis that can be obtained and maintained by the QC round strategy, samples with the lowest MRD levels can still represent a challenge: 17% (28/167) of our series resulted brd, showing discordant results in inter-lab assessments; 39% of them (11/28) remained brd even applying both methods. The results did not change even increasing the number of replicates/sample. Thus, although representing a minority, brd samples are still problematic, especially when a clinically oriented interpretation is required. As the combined use of standard methods does not totally solve this problem, alternative, novel, methods such as digital PCR and NGS need to be tested in this context.

**E1409**

**RHOA GLY17VAL MUTATION AND T-CELL CLONALITY ANALYSIS IN PATIENTS WITH ANGIOIMMUNOBLASTIC T-CELL LYMPHOMA**

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**Background:** Angioimmunoblastic T-cell lymphoma (AITL) is a rare subtype of T-cell lymphoma, characterized by generalized lymphadenopathy, hyperglobulinemia, and autoimmune manifestations. Interpretation of histological and immunohistochemical data can be difficult due to the small number of tumor cells, surrounded by abundant polymorphocellular infiltrate. AITL could often be misdiagnosed as reactive processes and other lymphomas, including Hodgkin’s lymphoma. T cell clonality assessment plays an important role in AITL diagnosis. However, ambiguous clonality results may be obtained. Recently discovered somatic RHOA Gly17Val mutation is present in 53-71% of angioimmunoblastic T-cell lymphomas. 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 morphological and immunohistochemical 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.

**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 TCRG and TCRB 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 morphological and immunohistochemical 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 Rs-0.8198, p-level <0.00001) between T-cell clonality (matching with LN clonal peaks) and the number of RHOA positive cells in the AITL samples (n 51). Skin, blood and bone marrow samples with the T cell clonality peaks that differ from those found in the LN were also negative for the presence of cells with RHOA (Gly17Val) mutation.

**Summary/Conclusions:** RHOA (Gly17Val) point mutation is detected in LN by allele-specific PCR in 60% of patients with AITL. The percentage of tumor cells in BM is low (averaging less than 2% of the total cells). However, combined molecular and histological data suggest that BM may be involved in most patients. Extent of T cell clonality (matching with LN clonal peaks) correlates with the amount of cells having a RHOA mutation. T-cell clonality in BM, skin, spleen, etc. with rearrangements not matching those identified for the LN should be considered reactive and possibly associated with autoimmune process or antiviral response.
Other Non-malignant hematopoietic disorders

E1410

USEFULNESS OF CHITOSIDASE ACTIVITY, CCL18/PARC, 7-KETOCHESTEROL AND GLUCOSYLSPHINGOSINE CONCENTRATIONS FOR SCREENING OF LYOSOMAL STORAGE DISORDERS

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening clinical syndrome due to a severe hyperinflammatory response. HLH is typically characterized by a very wide spectrum of clinical findings. Central nervous system affection (“CNS disease”) has been frequently described at presentation of HLH, during course of disease, or as isolated CNS-HLH that could precede other systemic clinical manifestations by months to years.

Aims: To study the value of CSF soluble interleukin-2 receptor alpha subunit (sIL2Rα) 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-2R Immunoassay, R&D Systems). Patients were considered as “CNS-HLH positive” when they had either neurological manifestations, abnormal findings on MRI or routine CSF analysis (elevated proteins &/or pleocytosis), and as “CNS-HLH negative” when they did not show any of these findings.

Results: We analyzed the data of 9 HLH patients; 4 females and 5 males. Their age ranged from 2 months to 13 years with a median of 5 months. Six patients had genetic diseases predisposing to HLH (Griscelli syndrome type II (GSI) & Chediak-Higashi syndrome (CHS)), and 3 other patients were diagnosed according to HLH-2004 diagnostic criteria and presumed to be of familial form (FHL). Out of the 9 patients, only 5 patients (55.6%) showed clinically evident neurological manifestations; 5 patients (55.6%) had elevated CSF proteins &/or pleocytosis, and 4 patients (44.4%) had an abnormal brain MRI. Overall 7 out of the 9 patients (77.8%) were “CNS-HLH positive” versus only 2 (22.2%) “CNS-HLH negative” according to classical criteria. Interestingly, the geometric mean of CSF sIL2Rα in CNS-HLH positive group was lower than in CNS-HLH negative group (734 vs 1952 pg/ml, p = 0.094). Moreover, CSF protein level and cell counts did not statistically correlate with CSF sIL2Rα level. Several patients showed interesting observations. Among the patients with statistically significant correlation between serum and CSF levels of sIL2Rα in our patients indicates that sIL2Rα is locally produced in the CSF secondary to cellular infiltration of the CNS and could be a valuable biological marker of disease activity. Larger prospective studies are warranted to confirm these results and determine diagnostic and prognostic value of CSF sIL2Rα levels, as well as its value for follow up of CNS disease.

E1412

GAUCHER DISEASE PATIENTS EXHIBIT A HIGH EXPRESSION OF LIPOPCINE (LCN2) AS POSSIBLE BIOMARKER OF RESIDUAL DISEASE ACTIVITY: AN EXPLORATORY STUDY AND CORRELATION WITH OTHER CYTOKINES

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Background: Gaucher Disease (GD) is characterized by a latent chronic inflammation/macrophage activation status expressed by an excessive pro-inflammatory cytokines, hyperferritinemia, hypergammaglobulinemia, altered calcium homeostasis and metabolic syndrome. Even patients under ERT do not fully revert this status and their risk to develop bone crisis, iron metabolism alterations, autoimmune disorders and neuropals remain higher. This observation to the creation of a novel auto-priming-regulatory auto -antigenic status. Monitoring of patients through chitotriosidase and CCL18/PARC has become essential however there are patients whom never normalize while others developed bone crisis/ complications after long-time under therapy and normal values. One of the key features for chronic inflammation is the anemia; this is characterized by hyperferritinemia in a common feature diagnosis of GD1 patients. Lipocine (LCN2), a cytokine released by adipocytes, mononuclear cells and neutrophils with expression on endothelial cells, hepatocytes and other cells, has been involved into the monocyte polarization and perpetuation of the inflammatory state. Based on this, we have performed an exploratory study assessing LCN2 expression in GD patients.

Aims: To explore the Lipocine (LCN2) expression as biomarker for disease activity in type 1 Gaucher Disease patients under different circumstances.

Methods: We have performed an exploratory study on 18 GD1 patients distributed in two cohorts. Cohort A was composed by 6 patients; 2 naïve (no prior enrollment to any type of therapy) and 4 under miglustat (M) therapy; this patient was part of the clinical study QUELAFER and were from baseline and after 4 months on chelation therapy were obtained. Cohort B included 12 patients on enzymatic replacement therapy (ERT), for this cohort sera samples were obtained for LCN2 determination and also a panel of cytokines (IL-10, IL-13, IL-4, IL-6, IL-7, Mip1α, Mip1β y TNFα), and hematocrit, hepcidin, cholinesterase and ferritin at the beginning of ERT and after one year on it. Data were incorporated into a database for this purpose including demographic and clinical available data. All patients have signed an informed consent for the use of their samples and ethical approved were obtained form institutional board of FEETEG foundation. A partial comparison comparing LCN2 expression vs the other biomarkers showed the patients showed increased levels of serum LCN2, the overall mean value for the initial sample was 171, 88 (66,72-261,72). As cohorts the differences among individual were significant (Cohort A, p=0.02 and cohort B, p<0.01).

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patients exhibit the higher values. In general 9 patients showed a reduction in LCN2 levels while 7 showed an increase and one the value was stable. All patients showed a reduction in ferritin and chitotriosidase, however a fully correlation with LCN2 expression were not founded. Globally there were no statistically significant differences between the two groups. Background: Thrombotic thrombocytopenic purpura (TTP) is a rare disease that is fatal if untreated. While the main treatment modality is plasmapheresis, immunosuppressants also play a crucial role in the treatment of TTP. Aims: Our aim is to compare the clinical characteristics, treatment and outcomes of patients with acquired primary TTP to those with secondary TTP (i.e. autoimmune and malignancy) hematopoietic stem cell transplant (HSCT) related. Methods: We reviewed all patients with TTP who received plasmapheresis at our institution from 1st Jan 2008 to 31st Jan 2017. Clinical and laboratory characteristics, treatment, response to treatment and complications were recorded. Complete remission (CR) was defined as platelet count normalization, partial remission (PR) as platelet count doubling and >30 x 10⁹/L, and the rest as unresponsive/mortality (UM). Results: Of 41 cases of TTP, 24% (n=10) was primary, 44% (n=18) was secondary to autoimmune diseases, 27% (n=11) was secondary to malignancy or HSCT, 5% (n=2) was related to DRESS syndrome and acute pancreatitis. The median age was 47 (18-86) years and it was predominately female (81%). About two-thirds of the cases presented with neurological symptoms (66%), renal dysfunction (56%) and fever (59%). Only 12 patients (29%) had TTP pentad. Proportionate to incidence of secondary TTP, 85% required immunosuppressive therapy. CR was seen in 51% (n=21) and PR in 15% (n=6). Mortality rate at end of treatment was 41% (n=17) and at end of follow-up was 46% (n=19). Comparison of demographics, clinical presentation, treatment and outcomes between acquired primary TTP and secondary TTP are shown in table below. 

Table 1. 

<table>
<thead>
<tr>
<th>Primary TTP</th>
<th>Acquired disease related TTP</th>
<th>Maligancy/HSCT related TTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>57 (30-96)</td>
<td>60 (20-92)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>Neurological symptoms (%)</td>
<td>98</td>
<td>72</td>
</tr>
<tr>
<td>Renal dysfunction (%)</td>
<td>98</td>
<td>72</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>98</td>
<td>72</td>
</tr>
<tr>
<td>Thrombocytopenia (median, range)</td>
<td>12.5 (3-84)</td>
<td>11 (4-24)</td>
</tr>
<tr>
<td>Anemia (%)</td>
<td>72</td>
<td>17</td>
</tr>
<tr>
<td>CR (CD3+)</td>
<td>60</td>
<td>72</td>
</tr>
<tr>
<td>Major Hematocrit change (%)</td>
<td>70</td>
<td>24</td>
</tr>
<tr>
<td>Days of hospitalization (median, range)</td>
<td>21 (10-60)</td>
<td>68 (5-100)**</td>
</tr>
<tr>
<td>CR: 30 days, range 15-103</td>
<td>60</td>
<td>17</td>
</tr>
<tr>
<td>PR (%)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Relapse (%)</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Follow-up duration in months (median, range)</td>
<td>32.2 (0.1-112)</td>
<td>32.2 (0.1-112)</td>
</tr>
<tr>
<td>Mortality at end of follow-up (%)</td>
<td>40</td>
<td>6 (0-100)</td>
</tr>
<tr>
<td>Days of hospitalization (median, range)</td>
<td>12 (10-22)</td>
<td>12 (10-22)</td>
</tr>
<tr>
<td>At completion of treatment</td>
<td>CRI (%)</td>
<td>60</td>
</tr>
<tr>
<td>CR: 30 days</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>PR (%)</td>
<td>20</td>
<td>20</td>
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<tr>
<td>Days of hospitalization (median, range)</td>
<td>12 (10-22)</td>
<td>12 (10-22)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Lipocaline expression is increased in GD1 patients in general, a correlation with other cytokines expression to establish the role of this biomarker is warranted.

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 100mg/kg PO/days 1-5. CR: hemoglobin (Hb) ≥12 g/dL, PR: Hb ≥10 g/dL or increase of ≥2 g/dL. Response was evaluated at day +28, months +6 and +12. Informed consent was obtained from all participants. Results: Eight patients were included. Median age was 32 years (range 18-42), 6 were female. Median Hb at diagnosis was 5.8 g/dL (range 4.8-8.2 g/dL). All patients had response at day +28 (50% CR rate); median time to response was 12 days (range 3-17). During follow-up 7/8 achieved CR (median time to CR: 30 days, range 15-103), all of which were sustained at 6 months. Median follow-up was 24 months (range 6-40). One patient relapsed disease-dependent and relapsed after 12 months, achieving a stable PR after re-treatment with low-dose rituximab. Furthermore, two patients had new-onset immune thrombocytopenia (IT; Fisher-Evans’ syndrome), without hemolysis 6.5 and 6 months
after diagnosis. Two patients were diagnosed with systemic lupus erythematosus during follow-up, they remained in CR. Twelve-month CR rate was 80% (5 evaluable patients). One patient experienced grade 3 neutropenia two months after the last rituximab infusion that resolved without complications. Estimated relapse-free survival was 80% at 2 years (60% if IT is considered). No patient had a splenectomy performed.

Summary/Conclusions: This small study reports favorable outcomes for patients with newly diagnosed wAIHA treated with low-dose rituximab, and adds 8 patients with similar responses to the 7 cases previously published by the Italian group in 2012 and 2015. These results may be comparable to standard doses of rituximab, with a lower cost, and deserves further inquiry. The emergence of additional autoimmune phenomena (SLE, Evans’ syndrome) is unpredictable and can be an obstacle for appropriate data analysis in prospective AIHA studies.

E1416
INFECTIOUS COMPLICATIONS IN PRIMARY AUTOIMMUNE NEUTROPHENIA OF CHILDHOOD
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Background: Primary autoimmune neutropenia (PAN) of childhood is caused by the action of antibodies against membrane antigens of neutrophils leading to their peripheral destruction. Despite the low neutrophil counts, it is characterized by minor intercurrent infections with rare severe bacterial episodes, which can be a significant cause of morbidity.

Aims: The retrospective evaluation of the incidence and characteristics of infectious complications in children with PAN from one reference academic center in Greece.

Methods: The study included the clinical and laboratory findings of children with PAN, who were diagnosed in our department in the last eight years (2008-2016). All children with neutropenia lasting over 3 months with a positive test for neutrophil antibodies, using the granulocyte immunofluorescence test, the granulocyte agglutination test and the monoclonal antibody immobilization of granulocyte antigen test. Laboratory evaluation for nutritional deficiencies, infections, systemic autoimmune diseases or malignancies was negative. Clinical data related to the occurrence of bacterial infections and treatment, hospitalization and outcome were collected and analyzed.

Results: 48 children with PAN were enrolled; 28 were boys, the median age was 14.5 months (range 5-96) and median follow-up time was 20 months (range 4-93). 19 children (39.6%) all suffering from severe neutropenia (<0.5 x 10^9/L) had to be hospitalized 25 times for bacterial infections; 4 for pneumonia, 7 for acute otitis media, 1 for mastoiditis, 7 for urinary tract infections, 4 for bacterial infections of unspecified site, 1 for perianal abscess and 1 for cellulitis, all with good outcome with proper antibiotic treatment. The average number of hospitalizations due to infections was 0,52/patient and the rate was 0.56/1000 patient-days. G-CSF was administered in 2 children due to severe infection, while 8 children received antibiotic chemoprophylaxis.

Summary/Conclusions: Although rare, infections are an important clinical issue in the management of children with severe PAN, sometimes requiring hospitalization. Early signs of infection should be promptly recognized and accordingly treated.

E1417
NEW EPO-RECEPTOR MUTATION IN A -17 YEAR OLD WOMAN WITH ERYTHROCYTOSIS
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Background: Erythrocitosis is defined when red cell, hematocrit (Hct) and hemoglobin (Hb), are elevated above normal limits. Causes of erythrocitosis can be primary and secondary. Secondary causes are divided into congenital and acquired. There is a group of patients with idiopathic erythrocitosis.

Aims: We present a case report of a novel EPO-Receptor mutation.

Methods: We present a case report of a 17-year-old woman with erythrocitosis. In the control blood test she had hemoglobin of 18.6g/dl and hematocrit of 62%. We contacted the patient and she attended hematology consultations for study and treatment with phlebotomy. The patient had no known drug allergies or toxic habits. She didn't have any known comorbidities or previous treatment. At evaluation she referred chronic headache without other symptoms. The physical examination was normal. At that time, three possible diagnose were suspected. Firstly, primary erythrocitosis, polycythemia vera (PV). In this disease, the bone marrow produces many red cells and the JAK2 V617F mutation has been demonstrated in the majority of patients. exon 12 mutation has been described in patients with PV who did not have the JAK2 V617F mutation. The erythropoietin (EPO) level is undetectable as a compensatory mechanism. In our patient, JAK2 V617F mutation and exon12 mutation were negative and the EPO levels were undetectable (<1.5). The bone marrow aspirate and the bone marrow biopsy were normal. These results show that this patient doesn’t present PV, due to she only fulfilling one diagnosis criteria of PV. Secondly, acquired secondary erythrocitosis can be produced as a compensatory mechanism, including: cardiac or pulmonary disease, smoking, renal artery stenosis, sleep apnea/hypopentilation and malignant tumors. In the patient, pulmonary function test, abdomen ultrasound and kidney function were normal. Endogenous erythrocytosis were positive. Due to the test results, we ruled out the diagnosis of acquired secondary erythrocitosis. Finally, congenital secondary erythrocitosis. Genetic abnormalities have been identified in congenital causes of erythrocitosis. The congenital erythrocitosis are divided into two sets according to EPO levels. If the EPO levels are normal or increased, the patient could present high oxygen-affinity hemoglobin because of bisphosphoglycerate mutase deficiency or mutations in the genes in the oxygen sensing pathway. However, if the EPO levels are decreased, the patient could present an erythropoietin-receptor mutation. Our patient presented undetectable EPO levels and the EPO-receptor mutation was requested. The patient has been treated with phlebotomies and aspirin due to headache with good evolution. In this moment, she presents hematocrit levels of 46.8%.

Results: The test revealed an EPO-receptor mutation (c.1275_1290dup), which had never been described before.

Figure 1.
Summary/Conclusions: The study of the patient with erythrocitosis must begin with a full medical history and confirmation of raised Hb and Hct. In the study of erythrocitosis, after ruling out primary and acquired causes we should always consider the possibility of congenital erythrocitosis, which often is underestimated. When EPO binds to its receptor a signaling cascade is activated, which cause red cells to be produced. This process is switched off when sufficient red cells have been produced by binding of SHP-1. EPO-receptor mutation results in failure of bond of SHP-1, causing uncontrolled production of red cells and erythrocitosis. We describe a new EPO-receptor (c.1275_1290dup) (figure 1).

E1418
FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHILDREN
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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder characterized with uncontrollable activation of T-helper lymphocytes and macrophages and over-release of inflammatory cytokines. The only curative treatment is hematopoietic stem cell transplantation (HSCT).

Aims: This study evaluates the clinical and laboratory data of children with FHL. Thirty five FEL cases followed and treated at our clinic between 2005 and 2017 were retrospectively evaluated in our study.

Methods: Information of patients were retrieved from patient files and from the records contained in the electronic information processing environment created after 2005. All patients were treated with HLH-2004 protocol. HSCT was performed in nine patients.

Results: Twenty one of the cases were boys and fourteen were girls. The age at presentation for patients was two week-three years (mean 6.2 months). There was a history of consanguineous marriage in 26 of the families(74%). Fever, anemia, and hypertriglyceridemia were present in all HLH cases. Hepatomegaly was detected in 74%. Splenomegaly was more frequent in patients (87.7%). Thrombocytopenia was detected in all patients. All patients had neutropenia and thrombocytopenia. Hypoferritinemia was present in 94% of patients. Only nine patients found to have hemophagocytosis in the cytological evaluation of the CSF(25.7%). Mutation analysis were performed in 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 opportunistic infection (n=10) or of disease progression (n=10).
Summary/Conclusions: In conclusion, FHL is a disease with high mortality rates and the only curative treatment is HSCT. Donor search for HSCT must be started and HSCT should be performed after the remission.

E1419
ABNORMAL MONOCYTE POPULATIONS IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA
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Background: Chronic idiopathic neutropenia (CIN) is an acquired disorder of granulopoiesis characterized by an unexplained, prolonged reduction in the number of neutrophils and a generally benign and uncomplicated course. Neutropenia in CIN has been mainly attributed to increased apoptotic death of the granulocytic progenitor cells due to abnormal production of pro-inflammatory cytokines and pro-apoptotic mediators. Activated T-lymphocytes with a skewed oligoclonal/monoclonal profile and myelosuppressive properties have also have a major role in the pathophysiology of CIN.

Aims: Monocyte subpopulations display a prominent role in innate immunity but also mediate pro-inflammatory responses and T-cell activation. The monocytic CD14+CD15-/DRneg/low/CD33+/CD11b+ derived suppressor cells (MDSC), in CIN patients.

Methods: We have studied 25 patients fulfilling the well-defined diagnostic criteria for CIN and 10 age and sex-matched healthy individuals. Three-colour flow cytometry was used to assess the peripheral blood monocytes subsets in the gate of CD14 positive cells and five-colour flow cytometry for the evaluation of the myeloid derived suppressor cells in the gate of cells with intermediate/high FSC/SSC properties.

Results: The mean number of neutrophils and monocytes in CIN patients was 1176±496/µl and 412±130/µl, respectively (range 200-1800/µl and 700-2000/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 non-classical CD14+CD16+ cells as well as the monocytic CD14+CD15/DRneg/low/CD33+/CD11b+ fraction of the myeloid derived suppressor cells (MDSC), in CIN patients.

Summary/Conclusions: CIN patients display increased proportion of circulating intermediate CD14+CD16+ monocytes that may have a role in the aberrant inflammatory responses commonly seen in these patients. The increased proportion of the CD14+CD15/DRneg/low/CD33+/CD11b+ MDSC in CIN may simply reflect a compensatory reaction aiming to suppress the T-cell activation. Isolation of the above cell populations and transcriptome studies are currently in progress in our laboratory.

E1420
DIAGNOSTIC VALUE OF CELL BOUND AND CIRCULATING ANTI-NEUTROPHIL ANTIBODY DETECTION IN PEDIATRIC NEUTROPENIA
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References

E1422
Abstract withdrawn.

E1423
RITUXIMAB IN AUTOIMMUNE HEMOLYTIC ANEMIA OF INFANCY
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Background: Autoimmune hemolytic anemia (AIHA) is not commonly seen during childhood, and is extremely rare in infancy. Absence of guidelines render management of the disease difficult in children – and even more so in infants.

Aims: Aim of the report is to present a number of cases of infantile AIHA, refractory to conventional treatments, demonstrating response in administration of rituximab.

Methods: The report concerns four infants (3 baby girls and one baby boy) who presented with AIHA. Data including demographics, personal and family medical history, immunologic assessments, previous treatments and response to rituximab were studied.

Results: Age at diagnosis of AIHA was 4-6 months. In 3 cases (cases number 1, 2 and 3) personal and family history, as well as laboratory screening at diagnosis, did not reveal presence of any other hematologic, autoimmune or immunologic condition. In case number 4 AIHA followed the diagnosis of giant cell hepatitis. Hospitalization before rituximab administration ranged between 1 and 5 months, with multiple transfusions, administration 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 hemolytic parameters were observed after the 3rd-4th weekly infusion in all infants. In 3 patients (no 1,2,3) CD19+ and CD20+B cell assessment before and after rituximab administration was performed. Complete elimination (<1%) was observed in all patients after the 1st-2nd infusion. Despite B cells returning to normal 11 months after treatment, infant no 1 remained in clinical remission during follow-up (22 months post treatment). Infant no 2 remained in clinical remission for the 16 month post treatment follow-up, despite B cell normalization. Infant no 3 relapsed following B cell normalization, 11 months after rituximab administration. Infant no 4 did not undergo B cell measurements and relapsed one year after completing rituximab therapy. The 2 patients that relapsed were re-treated with 4 rituximab infusions: patient no 3 remained well for the 18 month follow-up, whereas patient no 4 remained well for 10 years – again relapsing and receiving her 3rd rituximab treatment with good response for the remaining 7 month follow-up. None of the patients presented with adverse reactions during the infusions or with severities resulting in hospitalization. However, infant no 1 developed asymptomatic progressive IgG hypogammaglobulinemia 11 months after initial exposure to rituximab, eventually requiring IVIG administration.

Summary/Conclusions: Rituximab administration in refractory AIHA seems to be efficacious and safe in infants. However, close follow-up is warranted in order to ensure absence of long term complications, including the risk of post-treatment hypogammaglobulinemia, when the drug is administered at such young ages.

E1425
CONGENITAL ERYTHROCYTOSIS: DISCOVER OF A NEW MUTATION
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Background: Congenital erythrocytosis (CE) is a rare hereditary disorder of red cell production, characterized by an absolute increase in red cell mass with elevated hematocrit and hemoglobin levels not accompanied by increased oxygen affinity, and the absence of clinical symptoms. CE is a hereditary congenital disorder with a prevalence of 1 in 300,000 individuals. A total of 25 genes are known to be associated with CE. Most cases are due to mutations in the PHD2 gene which encodes prolyl hydroxylase domain 2 (PHD2), an enzyme involved in the control of EPO synthesis, by the HIF hydroxylation pathway.

Aims: To retrospectively analyze 44 patients diagnosed with CE in our center.

Methods: We performed whole-exome sequencing of 20 patients with a strong family history. A set of patients in whom targeted re-sequencing had been performed for the diagnosis of CE were included. In a further 22 patients, targeted re-sequencing of PHD2 and JAK2 genes was performed in order to exclude cases previously diagnosed and treated for myeloproliferative neoplasms.

Results: We identified a new mutation in PHD2 gene associated to CE. The mutation is a novel frameshift mutation (c.563_565del3, p.Leu188fs) located in the presence of oxygen, and the absence of 5p21.3 chromosomal region. This mutation was present in all probands from the same family and was co-segregated with the clinical manifestations of CE.

Summary/Conclusions: The presence of oxygen, and the absence of 5p21.3 chromosomal region. This mutation was present in all probands from the same family and was co-segregated with the clinical manifestations of CE.

E1426
A RETROSPECTIVE STUDY OF THE THROMBOTIC MICROANGIOPATHIES DIAGNOSED IN THE LAST 17 YEARS IN ONE SINGLE CENTRE
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Background: Thrombotic microangiopathies (TMA) are characterized by the formation of platelet thrombi that obstructs vital organ microcirculation. The presence of the 5 classic parameters (haemolytic anemia, thrombocytopenia, fever, oliguria and neurological affection) is rare. ADAMTS13 determination allows a more accurate diagnosis than the presumption based on clinical and biochemical parameters.

Aims: To retrospectively analyze 44 TMA patients diagnosed in our centre in the last 17 years and characterize TTP, HUS and secondary TMA (sTMA) by biochemical parameters.

Methods: We then proceeded to sequencing of gene included in EPO-induced signaling pathway and it was detected a new mutations in PHD2 gene (F366L), in heterozygous state. The mutation was detected in all probands from the same family and was co-segregated with the clinical manifestations of CE.

Summary/Conclusions: The mutation was detected in all probands from the same family and was co-segregated with the clinical manifestations of CE.

E1424
EARLY LESSONS FROM WHOLE-EXOME SEQUENCING IN THE CLINICAL DIAGNOSIS OF RARE AND UNRATIONALIZED 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 good resolution and sensitivity. Therefore WGS has the potential to offer an accurate molecular diagnosis in a proportion of unsolved anaemia cases and may therefore be a superior initial approach. Furthermore, WGS is likely to lead to the identification of novel genes involved in pathogenic and normal erythropoiesis.

Aims: To undertake WGS in a set of patients in whom targeted re-sequencing had not been able to identify a molecular cause for the inherited anaemia, in an attempt to increase the diagnostic yield of the molecular analysis of such patients and provide novel candidate genes as causative of anaemia.

Methods: We performed WGS of 20 individuals (2 siblings and 6 trios) at 30X coverage where the probands 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 Stampy for read alignment, Platypus for variant calling and Ingenuity Variant Analysis (Qigian) for variant annotation and filtering, followed by functional validation and verification of all candidate variants.

Results: Known causative variants in a gene absent from the targeted panel were detected in two patients (25%), whereas candidate variants in novel genes not previously associated with anaemia were identified across the other six cases. Familial segregation and functional studies are underway to provide further evidence of causality for these novel variants, of which 60% are in genes with previous evidence of a role in erythropoiesis and 40% in genes with no known role in erythroid development.

Summary/Conclusions: These results illustrate the overlap in phenotypic abnormalities existing among these conditions and the importance of providing an erythropoiesis and molecular diagnosis to enable correct diagnostic and clinical manage-ment 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.
CHILDREN WITH CHRONIC-REFRACTORY AUTOIMMUNE CYTOPENIAS: A SINGLE CENTER EXPERIENCE

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Background: Autoimmune cytopenias are a group of heterogeneous disorders characterized by immune-mediated destruction of one or more hematopoietic lineage cells. They can be idiopathic or occur as a manifestation of other underlying disorders, such as autoimmune diseases, immunodeficiency, autoimmune lymphoproliferative syndrome, tumors, medications or infections.

Aims: The aim of this study was to evaluate the clinical course and significance of autoimmune cytopenias due to immunodeficiency or autoimmune diseases in children followed up at our hospital.

Methods: A total of 337 files of information belong to patients with chronic or refractory autoimmune cytopenias were included in this study. Data were analyzed retrospectively at our hematology department between February 1997 and September 2015. Ultimately, patients with immune deficiency or autoimmune diseases (23 patients) were included in this study. Data were analyzed using SPSS 15.0. The results are presented as the mean, SD, median, absolute number, or percentile.

Results: Two-thirds of the patients with chronic or refractory 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 cytopenia was detected before the primary diseases were diagnosed after median 2 months (between 0 and 77 months). Only one patient firstly had been diagnosed as CVID, cytopenia has developed after years. All of the patients were treated with corticosteroids or intravenous immune globulin (IVIG) as first-line treatment. Ten patients needed second or further-line immunosuppressive therapies including rituximab, mycophenolate mofetil, cyclosporine A, azathioprine, and chloroquine. A total of 8 patients (34.7%) recovered from autoimmune cytopenias after the treatment of primer disease. That diseases were diagnosed as systemic lupus erythematosus in 4 patients, hypogammaglobulinemia in 3 patients, and celiac disease in 1 patient. Cytopenias have continued in 14 of the patients. One patient with CVID died.

Summary/Conclusions: Cytopenias may be the first finding of an immunodeficiency or autoimmune disease and primary may be diagnosed in the clinical course. Early diagnosis is important because of beginning to the early treatment of underlying disease.
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 FACS DIVA 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 49% persons at both time points. T-cell lymphopenia before HLH diagnosis was noted in 60% patients with myeloid malignancy but in only 25% cases of lymphoid malignancy, whereas in established HLH the respective figures were 27% and 46%. CD4/CD8 ratio was skewed-to-normal in both myeloid and lymphatic tumors before HLH was diagnosed. In cases of confirmed hyperinflammation, patients with myeloid tumors showed dominance of CD4+ cells but no such difference was noted in lymphoid disease. Loss of lineage specific markers of non-neoplastic T-cells was a constant feature in lymphoid malignancy, whereas aberrant expression of lymphatic markers (CD2, CD7, CD56) on myeloid cells was uniform in patients with myeloid tumors. Monocytosis was more often observed in myeloid as compared to lymphoid tumors 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 have an impact 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.

E1431

A MULTICENTRE, SINGLE ARM, OPEN LABEL STUDY EVALUATING THE EFFICACY AND SAFETY OF ERTOMBOPGAG IN PATIENTS WITH SEVERE PERSISTENT IMMUNE THROMBOCYTOPENIC PURPURA (ITP) WITHIN SIX MONTHS OF DIAGNOSIS

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Background: Patients with acute ITP who fail or are dependent on steroids or intravenous immunoglobulin (IVig) are often committed to splenectomy or pro- longed 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 count of ≥30x10^9/L despite a daily dose of prednisolone of 1mg/kg for at least 2 weeks from diagnosis OR (b) requiring prednisolone ≥10mg daily and/or recurrent doses of IVig to maintain a platelet of ≥30x10^9/L within 6 months of diagnosis. Prior splenectomy was not a requisite.

E1430

BLEEDING IN PRIMARY IMMUNE THROMBOCYTOPENIA: WHO ARE MOST AT RISK?

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Background: Primary Immune Thrombocytopenia is rare disorder in which patients are at risk of bleeding due to autoimmune-mediated platelet destruction. Aims: This study focused on describing the prevalence and types of bleeding events around the time of ITP diagnosis and after, as well as identify any factors that can potentially influence the risk of bleeding.

Methods: Data from the United Kingdom Immune Thrombocytopenia Registry were analysed for this study. The registry obtained its data from about 70 centres around the UK. Descriptive and logistic regression statistical techniques were used for this study.

Results: This analysis was based on 2365 (57.8% females) participants who are part of the Registry. The median age at diagnosis was 50 years (IQR 32, 66) and 77% of these patients were of European ethnicity. The commonest comorbid conditions was hypertension (23%). Median platelet count was 19 (IQR: 5, 53). Eighty percent had a platelet count below 30x10^9/L at ITP diagnosis. The most common bleeding events were skin-related (46.5%) and to the oral cavity (14.4%). About 70% of the cohort experienced at least one bleeding event at some point after diagnosis. After ITP diagnosis the most common bleeds were again skin-related (34.3%) and oral cavity bleeding (14.8%). Epistaxis had risen from 11.6% before diagnosis to 17.7%. Bleeding at other sites did drop. However, the prevalence of intracranial haemorrhage rose from 0.9% pre-diagnosis to 1.2% after diagnosis. Prednisolone (79%) and IVig (43%) were the most used drugs followed by rituximab (28%) among those who were treated. Ropiniplomist (15%) and Ertrombopag (9%) are used too but not anymore than mycophenolate (18%) and azathioprine (22%). Fourteen percent of the cohort had a splenectomy at some point. Age but not gender or ethnicity were found to be associated with having a bleeding event around the diagnosis of ITP. Both children (<18 years old) and adults (>70 years) who were at risk. Platelet counts, expectedly, was associated with bleeding with those presenting with a platelet < 30x10^9/L were at higher risk. No comorbid illness or cotherapies were found to be associated with bleeding events.

Summary/Conclusions: The frequency of bleeding decreased for most sites but for some others a slight increase has been observed since ITP diagnosis. It is possible that bleeding events may have been recorded more accurately or observed more closely and over a longer period of time since diagnosis. However, control of bleeding was an issue after the diagnosis of ITP. Future analysis stratifying its findings by time periods would be beneficial in describing if bleeding events were better controlled over the last few years, especially after the introduction of new therapeutic agents and the publication of the internal consensus report on the diagnosis and management of primary ITP.

E1429

FLOW CYTOMETRIC ANALYSIS OF TISSUE SAMPLLES IN 42 ADULT PATIENTS WITH MALIGNANCY-ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Results: Paired BM samples were available. Clones of myeloid or lymphoid character were excluded from reanalysis for the purpose of this study. Bone marrow 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 FACS DIVA software. Neoplastic clones of myeloid or lymphoid character were excluded from reanalysis for the purpose of this study. Bone marrow samples were obtained from 31 patients shortly before and from 24 patients following HLH-diagnosis; in 13 patients paired BM samples were available.

Results: Patient characteristics are presented in table 1. Bone marrow B-cell lymphopenia was observed in 67% patients before and 74% after HLH diagnosis. Decreased amounts of NK-cells were noted in 49% persons at both time points. T-cell lymphopenia before HLH diagnosis was noted in 60% patients with myeloid malignancy but in only 25% cases of lymphoid malignancy, whereas in established HLH the respective figures were 27% and 46%. CD4/CD8 ratio was skewed-to-normal in both myeloid and lymphatic tumors before HLH was diagnosed. In cases of confirmed hyperinflammation, patients with myeloid tumors showed dominance of CD4+ cells but no such difference was noted in lymphoid disease. Loss of lineage specific markers of non-neoplastic T-cells was a constant feature in lymphoid malignancy, whereas aberrant expression of lymphatic markers (CD2, CD7, CD56) on myeloid cells was uniform in patients with myeloid tumors. Monocytosis was more often observed in myeloid as compared to lymphoid tumors 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 have an impact disease-specific impact on BM microenvironment. Further study will be carried out to confirm findings in a large, possibly prospectively collected patient group. Control group of patients with respective malignancies but without HLH will be included.
Patients with platelets <10x10^9/L will commence on eltrombopag at 75mg daily; 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 dose can be progressively weaned to zero over the subsequent 6 weeks if clinically appropriate. The primary endpoint was overall response rate (ORR) at week 12, defined as the proportion of patients achieving complete response (CR; platelet >100x10^9/L), partial response (PR; platelet >50x10^9/L) or minor response (MR; platelet 30-50x10^9/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 81 (74, 87) years, and median (Q1, Q3) time from diagnosis to ITP/AML diagnosis was 2.2 (1, 5.4) months, and median (Q1, Q3) screening platelet count was 21(13, 34) x10^9/L. Prior treatments included steroids (95%), IVIG (58%), and immunosuppression (28%). 35 patients (90%) completed 12 weeks of treatment, 4 (10%) discontinued eltrombopag prior to week 12 [3 required new ITP therapy; 1 developed thrombotic microangiopathy at platelet 97x10^9/L; one pulmonary embolism at platelet 240x10^9/L). At week 12, the ORR was 64% (p<0.0001; 90% CI: 51-77%); CR, PR, MR rates were 41%, 15%, and 4% respectively and the median (Q1, Q3) platelet count among responders was 168 (98, 252)x10^9/L. At week 26, the ORR was 54% (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/Discussion:** 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.
NORDIC COUNTRY PATIENT REGISTRY FOR IMMUNE THROMBOCYTOPENIA (NCPaITP): A COHORT OF PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA IN DENMARK, SWEDEN, AND NORWAY

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Background: Immune thrombocytopenia (ITP) is a rare disease characterized by isolated 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.

Methods: Encompassing Denmark, Norway, and Sweden, the NCPaITP started as a population-based post-authorisation 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 and a history of 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 national health registries 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 thrombotic/thrombotic events). Additionally, available bone marrow samples are maintained and reexamined for reticulin and collagen content to assess Thiele’s myelofibrosis (MF) grading.

Results: The NCPaITP 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 <50×10⁹/L. 18% were splenectomized, and 41% had at least one previous ITP therapy (mainly oral glucocorticoid steroids). The majority (68%) of the cohort had no underlying conditions included in the CCI at study enrollment, but 8% had a CCI score of 3 or higher, indicating severe comorbidity. Of note, based on hospital diagnoses of specific comorbidities recorded within 5 years before study enrollment, 12% had a history of stroke, 9% had diabetes, and 18% had a history of hypertension. Currently, 718 bone marrow samples from 566 patients have been retrieved.

Summary/Conclusions: The NCPaITP provides an example of how, within the Nordic countries’ uniform health care systems, registries can be established to study the clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.

E1436

EPIDEMIOLOGY OF PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) IN ADULTS IN RUSSIAN FEDERATION (RESULTS OF REGISTRY OF NATIONAL HEMATOLOGIC ASSOCIATION)

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Background: Immune thrombocytopenia in adults in Russia.

Methods: To describe the establishment of the NCPaITP and the characteristics of patients enrolled.

Aims:

1. Standartisation of methods of therapy, National Research Center for Hematological Hospital, Amursk, 14
2. Hematology, Regional Clinical Hospital, Khabarovsk, 14
3. Department of Medicine, Østfold Hospital Trust, Fredrikstad, Norway, 4
4. Center for Observational Research, Amgen Inc, San Francisco, 5
5. Center for Observational Research, Amgen Inc, Thousand Oaks, United States

Results:

1. 566 patients have been retrieved.
2. 8% had a history of a solid tumor, 9% had a history of diabetes, and 18% had a history of hypertension. Currently, 718 bone marrow samples from 566 patients have been retrieved.

Summary/Conclusions:

1. The NCPRaITP provides an example of how, within the Nordic countries’ uniform health care systems, registries can be established to study the clinical course of rare diseases such as ITP and the safety of drugs used to treat these patients.

E1437

ELTROMBOPAG (EPAG) FOR THE TREATMENT OF PATIENTS AGED ≥65 YEARS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (CITP): SAFETY AND EFFICACY RESULTS FROM THE EXTEND STUDY

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Background: ITP is an acquired autoimmune disorder characterized by isolated platelet count reduction, which is chronic when present for >12 months. Evidence suggests that age may influence both the hemorrhagic manifestations of ITP and also response and adverse events (AEs) associated with some therapies. Changes in drug metabolism can contribute to increased AE rates in patients (pts) ≥65 yrs compared with younger adults. The oral thrombopoietin-receptor agonist, EPAG, is approved for the treatment of previously treated (eg corticosteroids, immunoglobulins) CITP pts, but limited data are available in pts ≥65 yrs old. The EXTEND study was a global, open-label, extension study that evaluated long-term efficacy, safety and tolerability of EPAG in adults with cITP who had participated in prior EPAG studies.

Aims:

1. To describe the efficacy, durability of response, and safety of EPAG use in pts with cITP aged ≥65 yrs.

Methods:

1. 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 ≥50–200×10⁹/L. Maintenance dosage con- nected to individual platelet count response or minimization of CITP 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.

Results:

1. At baseline (BL), 50/302 pts (17%) on EXTEND were ≥65 yrs old. At BL, 50 (50% of 50 yrs) achieved ≥50×10⁹/L, 24 (48%) were splenectomized. 52% were female and 74% had platelet counts <30×10⁹/L. Twenty-four pts (48%) withdrew early from the study, most commonly because of AEs (n=8, 16%), other reasons (n=7, 14%) and lack of efficacy (n=5, 10%). Median exposure duration was 2.3 yrs (range, 2 days to 7.9 yrs) and mean daily dose was 49.9 (range, 11–74 mg). In general, 43 (86%) pts achieved platelets ≥50×10⁹/L without rescue therapy; 37 (74%) achieved platelets ≥50×10⁹/L for ≥50% of assessments. 26 (52%) maintained platelet counts continuously ≥50×10⁹/L for ≥22 weeks (Fig. 1).

Medication time maintaining platelet counts >50×10⁹/L and twice BL values,

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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 yr (15%). AEs were reported in 47 (94%) pts, most frequently nasopharyngitis (n=13, 26%), constipation (n=12, 24%), fatigue (n=12, 24%), diarrhea, arthralgia, urinary tract infection, cataract and cough (all n=11, 22%). Serious AEs occurred in 24 (48%) pts, most frequently >5% cataracts (n=7, 14%), pneumonia (n=4, 8%) and urinary tract infection (n=3, 6%). The most frequent AEs with suspected relationship to study drug were cataracts (n=4, 8%), headache, fatigue, and increased ALT, AST and bilirubin (all n=3, 6%).

Figure 1.

Summary/Conclusions: The efficacy of EPAG in cITP pts >65 yrs was consistent with that seen with the overall EXTEND study population (Bussel et al. Haematologica 2016;111[1]:SS17), with sustained platelet increases and reduced bleeding. EPAG was well tolerated; AE rates were similar to that reported in the overall EXTEND study population, but an apparent increase in cataracts was observed in pts >65 yrs old (cataract incidence was 7% and 22% in <65 and >65 age groups, respectively). Further outcomes in patients >65 yrs old will be presented. Results should be interpreted with caution as almost half of the pts withdrew from the study. EPAG is an effective treatment option for certain cITP pts >65 yrs; its use should incorporate baseline cataract screening and regular monitoring.

E1438

SAFETY AND EFFICACY OF THROMBOPOIETIN RECEPTOR AGONISTS IN PATIENTS WITH PREVIOUSLY TREATED CHRONIC IMMUNE THROMBOCYTOPENIA: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Background: The current American Society of Hematology guideline recommends the use of thrombopoietin receptor agonists, eltrombopag or romiplostim as the second-line therapies for chronic immune thrombocytopenia (ITP). The safety and efficacy of those drugs have been tested in several clinical trials. However, the safety profile was not consistent throughout trials and is not yet well understood.

Aims: We herein conducted a meta-analysis of randomized controlled trials to compare the safety and efficacy of thrombopoietin receptor agonists: eltrombopag or romiplostim versus placebo in patients with previously treated chronic ITP. Our primary outcome was drug-related adverse events greater than CTCAE grade 3.

Methods: We performed a literature search in MEDLINE, EMBASE, Cochrane library, and the American Society of Hematology website up to September, 2015 by two independent authors according to PRISMA guideline. We included only randomized clinical trials comparing eltrombopag or romiplostim versus placebo. Random-effects model was used to estimate pooled Odds Ratio (OR). Results: A total of eight trials including 834 participants were included in the analysis. There was no significant difference of grade 3 or higher adverse events, such as thromboembolism, this meta-analysis showed that thrombopoietin receptor agonists are safe, well-tolerated, and effective in patients with previously treated chronic ITP.

E1439

CHILDHOOD IMMUNE THROMBOCYTOPENIA: A NATIONWIDE COHORT STUDY ON CONDITION MANAGEMENT AND OUTCOMES

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Background: Little is known about the management of pediatric ITP in real life, that is, routine clinical practice. Moreover, the predictive value of these factors upon disease outcome was explored individually and therefore the confoundment of other cell lineages 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 7x109/L, and 214 (81%) patients initiated medication. Factors independently associated with treatment initiation included platelet counts <10x109/L (p<0.0001) and mucocutaneous bleeding symptoms at baseline (p<0.001). At 12 months, data were available in 211 (82%) children, of whom 130 (74%) had recovered. Predictors of chronicity included female gender (OR=2.2; 95% CI=1.0–4.8), age ≥10 years (OR=2.6; 95% CI=1.1–6.0) and platelet counts ≥10x109/L (OR=3.2; 95% CI=1.5–6.9).

Summary/Conclusions: In routine clinical practice, the decision to apply a watchful-waiting strategy seems to be driven by platelet counts even in the absence of bleeding symptoms, resulting in treatment being initiated in more than 80% of the children surveyed. Overall, younger children with ITP showed good prognosis, with lower platelet counts and, to a lesser extent, male gender predicting more favorable outcomes.

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 syndromes2.

Aims: The aim of this study is to evaluate the outcome and toxicity of patients with ITP either primary or secondary to ALPS-like syndromes, with or without 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 ≥50x109/L and >30x109/L and at least 2 fold increase of the baseline count, respectively.

Results: 23 children aged 0-12 yrs (median 6) with primary ITP (7) or secondary to an ALPS-like disorder (16), were treated with Sirolimus. Seven patients (30%) with ALPS-like also had an Evans syndrome (ES), due to the association of leukopenia (1), or to the presence of trilinear cytopenia (6). Four patients with ALPS-like were found to have mutations on PIK3CD, CTLA4, TACI, and CARD 11 gene. All patients, but one treated in first-line, received Sirolimus as second (4), third (14) or fourth (4) line treatment, respectively. 18 patients had previously failed Mucocidential leptomeningeal MFME therapy. Overall, 17/23 (74%) patients achieved a response that was complete and partial in 12 (52%) and 5 (21%) patients, respectively. 10/23 patients were treated in 67 (86%) cases. Children with mono-linear ITP achieved a response in 11/16 (68%) cases, in particular 4/7 (57%) and 7/9 (77%) patients with primitive or secondary disease, respectively. 12 out of 18 (66%) patients who failed MFME therapy responded to Sirolimus rescue. Three patients (13%) reported toxicity consisting of cutaneous (2) and gastrointestinal issues (1) that required the interruption of the treatment in 2 cases.

Summary/Conclusions: To the best of our knowledge this is the largest cohort of patients with ITP or ES -other than ALPS -treated with Sirolimus, that showed to be safe and effective in most cases, including patients who previously failed
MMF treatment. Therefore, it can be considered as an alternative therapeutic option in the setting of ITP non only for patients with an underlying diagnosis of ALPS but also for the ones with primitive disease or with an ALPS-like disorder.

References

E1441
ASSESSMENT OF ROMIPLOSTIM SELF-ADMINISTRATION BY PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA AND CAREGIVERS FOLLOWING RECEIPT OF HOME ADMINISTRATION TRAINING (HAT) MATERIALS: A PRELIMINARY STUDY
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Background: A HAT pack was designed as an additional risk minimization tool to support healthcare providers (HCPs) in selecting patients and training of patients/caregivers to mitigate medication error risk when self-administering romiplostim subcutaneously, a thrombopoietin-receptor agonist which is approved in the European Union (EU) to treat chronic immune thrombocytopenic purpura (ITP) refractory to other treatments.

Aims: To estimate the proportion of adult patients and caregivers who administered 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 subsequent 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/cmm) 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) gene with Primary Immune thrombocytopenia (ITP).

Methods: Genotyping for the FCγIIA 131 H/R (A>G) was performed using polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) in 70 ITP patients and 70 healthy controls.

Results: The mean age of patients and control was 29.5±13.86 yrs and 27.90±8.89 yrs respectively. Male/Female ratio in patients and control was 1:2. Under additive model, the heterozygous genotype (AG) of the FCγIIA 131 H/R (A>G) polymorphism shows the significant association with ITP, (Odds Ratio 2.14 (95% CI, Lower: 1.07 Upper 4.29 P-value 0.0328)). Under dominant model, the Odds Ratio was 2.42 (95% CI, Lower: 0.34 Upper 0.94) with the significant P-value 0.014). 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 0.297)).

Summary/Conclusions: The study shows the association of heterozygous genotype (AG) of FCγIIA 131 H/R (A>G) with ITP. The dominant model also shows significant association with ITP. We conclude that mutant allele (G) in FCγIIA 131 H/R (A>G) gene polymorphism may have impact on susceptibility to ITP.
follow-up was 102±10 x10^9/L (range 54-336). Disease duration of less than 3 mos prior to therapy start was associated with better outcome (log rank p=0.049, Fig 2) with a median RFS not reached; median RFS for pts treated after 3 mos of diagnosis was 31 mos [OR: 3.8 (CI 95% 0.9-16.3), p=0.0067]. No significant association between gender (p=0.67), age at treatment (more or less than 60 yrs) (p=0.89), DTX total dose (more or less than 480mg) (p=0.35) was found. Summary/Conclusions: Pulsed HD-DXK is a well tolerated and highly effective first line treatment for TTP in every daily clinical practice. The role of a reduced-dose schedule needs to be explored in a larger cohort of pts. Treatment of newly diagnosed ITP pts - i.e. within 3 mos of diagnosis (Rodeghiero Blood 2007) - seems to lead to longer RFS.

E1444

EFFECT OF OSELTAMIVIR TREATMENT ON PLATELET COUNTS

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Background: As platelets lose sialic acid during aging and circulation, they are cleared by the hepatic Ashwell-Morell receptor (AMR) (1). A recent study suggests that inhibition of sialidase by oseltamivir, a commonly administered anti-influenza medication that inhibits viral sialidase, could associate with an increase in platelet counts (2).

Aims: The aim of this study was to analyze the effect of oseltamivir treatment in platelet counts.

Methods: We performed a retrospective single-center study. From November 2009 until March 2015, a total of 168 patients from our Hematology Unit were prescribed oseltamivir due to clinical suspicion of influenza. A total of 120 patients were excluded because they had received myelotoxic chemotherapy within 30 days (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 transplant recipients). We observed a significant increase in the mean platelet count after treatment with oseltamivir (170/95 x10^9/L vs 192±103 x10^9/L, p=0.04). As in the previous study (2), this effect was independent of whether influenza was diagnosed (Table 1). In addition, we did not discern significant fluctuation in platelet counts when treatment was immediately interrupted after a 30-day time lapse (184±100 x10^9/L vs 182±91 x10^9/L).

Table 1.

<table>
<thead>
<tr>
<th>Platelet count (x10^9/L)</th>
<th>in patients with a clinical suspicion of influenza</th>
<th>and after oseltamivir treatment (median of 5 days)</th>
<th>(n=44)</th>
<th>results are given</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before treatment</td>
<td>after treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100±90 x10^9/L</td>
<td>120±103 x10^9/L</td>
<td>148±103 x10^9/L</td>
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<td>130±100 x10^9/L</td>
<td>140±100 x10^9/L</td>
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</tr>
<tr>
<td>160±100 x10^9/L</td>
<td>200±100 x10^9/L</td>
<td>230±100 x10^9/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180±100 x10^9/L</td>
<td>210±100 x10^9/L</td>
<td>260±100 x10^9/L</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 with un-matched, raised troponin in a high risk factor for new cardiac events 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. Management of 34 patients underwent cardiac MRI scanning between September 2008 and November 2014 whilst being treated for an acute episode of immune mediated TTP. All patients had troponin-t measurement on admission and a transthoracic echocardiogram within 72 hours of presentation. All patients were treated for their TTP episode with plasmapheresis, steroids and Rituximab. Two cardiologists reported each MRI scan and only agreed, unequivocal findings were considered.

Results: The median age of patients was 49 (range 13-73), 71% of whom were women. Two patients had a diagnosis of hypercholesterolemia prior to TTP diagnosis but otherwise there was no previous cardiac history. 71% of patients had a raised troponin-t at presentation (normal <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 cardiac arrest. There was myocardial ischaemia in 23% of patients, with an abnormal T-wave pattern in those with an abnormal cardiac MRI results. Late gadolinium enhancement (LGE) in the mid-apical regions, a finding suggestive of focal myocardial fibrosis and usually secondary to myocardial necrosis, was seen in all 8 patients, subendocardial LGE in six, transmural LGE in one and one patient had both subendocardial and transmural LGE. The latter patient was the only one with an abnormal scan seen on the preceding 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-t was not in those with an abnormal normal cardiac MRI (MRI normal 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.
group and 56 (24-76) in the control group. Overall MEFV mutation prevalence was %25.9 (21/81) in the study group and %24.7(46/186) in the control group, (p=0.963). MEFV mutation distribution prevalence was similar in both gender groups among ITP patients and their presence did not alter the age of disease onset, (p>0.05). Similarly, presence of mutations did not change the platelet count at diagnosis, the number of treatment courses, the rate of patients undergoing splenectomy and primary steroid resistance. Although statistically not significant, there was a trend towards a better overall response to steroids in patients carrying MEFV mutations, %94.7 vs%82.8, (/p=0.28) respectively. The median time to loss of response to steroids was 60 (10-124) months in patients with mutations and 42 (19-24-68) months in patients without MEFV mutations, (p=0.004). The median time to splenectomy was 101 (42.5-159.5) months in the MEFV mutation carriers and 51 (46-56) months in the non-carriers, (p=0.48). Time to loss of response to splenectomy was 38 (12-90.9) months in mutation carriers and 54 (14-9.3-1) months in non-carriers, (p=0.42).

Summary/Conclusions: To the best of our knowledge, our study is the first to address a possible effect of MEFV mutations on MEFV mutation carrier rates were similar in both ITP and control groups. Although MEFV carrier state had no affect on clinical features of ITP, mutation carriers tended to have a better overall response to steroid treatment, stayed longer in remission, had a longer time to splenectomy and relapsed earlier after splenectomy.

E1447
PD-1 AND CTLA-4 POLYMORPHISMS AFFECT THE SUSCEPTIBILITY AND CLINICAL FEATURES OF CHRONIC IMMUNE THROMBOCYTOPENIA
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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 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, -72909C/T, A215V) and 4 CTLA-4 SNPs (-1577A/G, -1577G/A, +49AA, +49GG) 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 study was approved by the Institutional Review Board of Gunma University Hospital, Maebashi, Gunma, Japan.

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 42 (31.0%) were corticosteroid independent. Patients compared to healthy controls, the higher frequency of PD-1 -72909 TT genotype (low produc- er) 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 -72909 TT genotypes (low producer) was significantly associated with high frequency of treated patients, treated patients with corticosteroid, and steroid-dependent patients compared with CC & GT genotype (high producer) (94.4% vs 71.5%, 94.4% vs 57.7% and 52.9% vs 23.6%; /p=0.043, 0.003 and 0.188, respectively). On the other hand, CTLA-4 -49AA genotype (high producer) was significantly associated with low bleeding tendency than AG & GG genotype (low producer) (27.3% vs 63.8%, /p=0.017). CTLA-4 -1577 AA genotypes (high producer) was significantly associated with low bleeding tendency and steroid treatment than AG & GG genotype (low producer) (27.3% vs 63.8%, /p=0.017). CTLA-4 -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).

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.
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 period from diagnosis to the last follow-up visit of their last treatment. The median (Q1, Q3) time from ITP diagnosis to romiplostim initiation was 21.7 months (4-85 months) in the FAS. 123 FAS patients received prior ITP therapies; most of them received corticosteroids (104 [75.9%]). 117 patients (85.4%) were non-splenectomized before romiplostim therapy, for reasons such as refusal of splenectomy, comorbidities, or age. Over the observation period, romiplostim was injected at a median (Q1, Q3) dose of 3.11 mcg/kg/bw (1.8-4.8) FAS over a median (Q1-Q3) treatment period of 103 weeks (33-104). The median platelet count rose sharply from baseline (29.0 x 10^9/L) to two weeks of treatment (62.5 x 10^9/L). From week 3 to the end of the treatment period, the platelet count remained in a range of 50 x 10^9/L and 145.5 x 10^9/L. Since the start of the romiplostim therapy, 59 patients out of 137 (43.1%) received concomitant therapies, mostly corticosteroids (49 patients [35.8%]). The overall number of ADRs was 112 in the FAS, affecting 57 patients (27.0%). The most frequent ADRs were gastrointestinal (10.2%) and neurological (11.7%) ADRs, followed by constitutional symptoms (10.8%). Adverse drug reactions pertaining to blood/bone marrow affected 2.9% of patients (vascular/thrombotic events, bone marrow fibrosis), whereas bleeding as an ADR was seen in 0.7% of patients. The exposure-adjusted rate of bleeding events (grade 3 or 4) per 100 patient-years 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 study.

Summary/Conclusions: This study of routine clinical practice in Germany showed that treatment with romiplostim in ITP patients resulted in a rapid increase in platelet counts to levels maintained between 50 and 250 x 10^9/L over time, regardless of the splenectomy status of the patients; most of them were non-splenectomized. The product was well tolerated and achieved a decrease in the rate of ITP-related hospitalization.

E1450
THE CLINICAL UTILITY OF NEUROPSYCHOLOGY TESTING IN IMMUNE MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA
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Background: It is well recognized that neurological manifestations are common in thrombotic Thrombocytopenic Purpura (TTP) however research into the neuropsychological impact of the disease is scarce despite 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 thrombocytopenic purpura.

Methods: Between January 2010 and 2015, all patients within a single tertiary hematology center with a confirmed diagnosis of TTP were reviewed as outpatients (the full analysis set, FAS), 102 of them completed the 2-year observation period. The most common symptom leading to neuropsychology assessment was problems with concentration, experienced by 89% of patients. 44% had problems with memory, 39% felt depressed and 33% had anxiety issues.

Results: Between the two years of the study period, 56% were Caucasian, 33% Afro-Caribbean and 11% of South Asian origin. 16-67 years (mean 43.7, SD 12.1, range: 18-67). 50% (n=6) had mild cognitive impairment, 33% (n=6) mild-moderate impairment and 11% (n=2) severe impairment. The most common symptoms leading to neuropsychology assessment were problems with concentration, experienced by 89% of patients. 44% had problems with memory, 39% felt depressed and 33% had anxiety issues.

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.
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), HELLP syndrome, and to correlate this with evidence of in vivo coagulation activation using D- dimers.

**Methods:** Plasma samples from patients with EOP were accessed from a clinical biobank. Platelet activation markers were characterized using ELISA assays measuring platelet factor 4 (PF4), soluble glycoprotein VI (sGPVI) and neutrophil activating peptide-2 (NAP-2). Platelet microparticles (CD42a+ microparticles) were measured by flow cytometry. Platelet activation biomarker levels were adjusted by platelet count and expressed as /10⁸ platelets/ml. All data was analysed using GraphPad Prism 7. Parameters were reported as means±SEM.

**Results:** Plasma samples from 19 individual patients were included. Patients with HELLP syndrome demonstrated significantly greater numbers of CD42a+ microparticles when corrected for platelet count compared with those without HELLP syndrome (598x10⁹ ± 220x10⁹ versus 297x10⁹ ± 37x10⁹, p=0.04). Similarly, patients with HELLP syndrome demonstrated increased levels of sGPVI than those without HELLP; corrected for platelet count (2.576 ± 0.967 versus 1.22 ± 0.124 ng/10⁹ platelets/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±10.747 μg/ml compared with non-severe patients 1.85±2.350 μg/ml (p=0.537, Spearman R correlation coefficient, r=0.27, p=0.04).

**Summary/Conclusions:** The results of this study demonstrate a positive correlation between severity of pre-eclampsia and platelet activation, as measured by levels of platelet-derived microparticles and platelet GPVI expression. A number of anti-thrombotic and anti-platelet therapies have evaluated the role of low-dose aspirin therapy as prevention for pre-eclampsia, and there is Grade 2B evidence for its use in those at risk of severe pre-eclampsia. The evidence of enhanced platelet activation in our study provides rationale for the efficacy of aspirin in this setting, and the potential for novel antithrombotic agents to be studied for the same indication.

**E1454 PRIMARY ITP IN ADULTS TREATED WITH ELTROMBOPAG: A RETROSPECTIVE STUDY USING DATA FROM THE UNITED KINGDOM ADULT IMMUNE THROMBOCYTOPENIA REGISTRY.**

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²Haematology, Novartis, Camberley, Surrey, United Kingdom

**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. The 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 dose used, number of prior therapies, median length of treatment with eltrombopag, median counts at baseline before treatment and at 6 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 (65%), 9% between 2011 and 2013 and 9% between 2017 and 2018. The median platelet count at eltrombopag initiation was 97x10⁹/L (2-739). Patients who did not interrupt treatment are sustained remission after discontinuation. The median platelet count at suspension of Romiplostim was 91.5x10⁹/L (3-320). In patients treated with Eptifibatide 16 achieved a complete response, 5 a response, obtaining response in the 80% of cases; 5 were no responders. 14 (53%) patients stopped Eptifibatide after a median time of 1.5 months (1-6) for adverse events (2 cases of major cardiovascular events, 1 of myeloid/myelodysplasia, 1 of neurotoxicity, 2 of major bleeding, and 1 of venous thromboembolism). 13/26 patients who received Eptifibatide were at the 3rd line of treatment, 1 at the second, and the others were at least at the 4th line. The median platelet count was 21x10⁹/L (3-52) at the start of Romiplostim, with a median starting dose of 1 µg (1-2) and 17x10⁹/L (1-53) in patients treated with Eptifibatide, with a median starting dose of 50mg (25-50).

**Results:** Patients treated with Romiplostim we observed 22 complete responses and 10 responses, with a 82% response rate, while 7 patients were no responders. In our study 26 (66%) patients stopped Romiplostim after a median time of 16 months (1-93): 9 for stable response, 5 for no response, 3 for loss of response, 3 for adverse events (2 for bone marrow fibrosis, 1 for headache), 2 for major bleeding, 1 for loss of response associated to visual disturbances and gastrointestinal disorders, 2 underwent splenectomy, and 3 patients interrupted the treatment for other causes (es. diagnosis of cancer). The median platelet count at suspension of Romiplostim was 91.5 x10⁹/L (3-320). In patients treated with Eptifibatide 16 achieved a complete response, 5 a response, obtaining response in the 80% of cases; 5 were no responders. 14 (53%) patients stopped Eptifibatide after a median time of 1.5 months (1-12): 6 for adverse events (2 cases of major cardiovascular events, liver toxicity, skin rash, pharyngitis), 5 for no response, 1 for loss of response, 2 patients who achieved a CR interrupted Eptifibatide obtaining a sustained remission after discontinuation. The median platelet count at suspension of Eptifibatide was 13.7x10⁹/L (7.2-29x10⁹/L). In patients who did not interrupt treatment, a median time of 29 months (3-96). Several studies report- ed Romiplostim and Eptifibatide to be highly effective against chronic ITP, with average immediate responses exceeding 80% in our study. We observed that therapeutic response was influenced by the starting platelet count. In particular platelets count before therapy influenced the first response observed. In particular in patients treated with Romiplostim only PR patients directly correlated with the first response and the maintainance of response during treat- ment at month 1°, 2° 3° and 6. Patients with a median starting platelet count of 15x10⁹/L obtained a response (CR + R), while almost all patients who started therapy with PLT<15x10⁹/L at baseline can obtain an initial response, but the majority is non-responder.

**Summary/Conclusions:** TPO-mimetics have proved efficacy in patient with ITP and their use can be applied in several conditions (bridge to splenectomy; sustained response; switch and discontinuation). Future study on large series of patients are needed to test common baseline platelets with hematological and clinical characteristics. The two currently available agents (Romiplostim and Eltrombopag) have similar efficacies and only slightly different safety profiles, being effective in restoring a safe platelet count in 70%-80% of cases with chronic ITP failing one or more lines of treatment, including splenectomy.
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 and multivariate analysis.

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>ITP with NO response to oseltamivir</th>
<th>ITP with response to oseltamivir</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>78.5 (79.8-79.5)</td>
<td>49.5 (46.7-52.3)</td>
</tr>
<tr>
<td>Sex</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Platelet count (x10^9/L)</td>
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<td>After</td>
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<tr>
<td>Vaccination</td>
<td>Before</td>
<td>After</td>
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<tr>
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<tr>
<td>Steroids</td>
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<tr>
<td>Splenectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
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<tr>
<td>RGA-1 expression in patient platelets before OT (ratio)</td>
<td>Increase in RGA-1 expression in patient platelets after OT (ratio)</td>
<td></td>
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<tr>
<td>RGA-1 expression in control platelets, patient sera, before OT (ratio)</td>
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<td></td>
</tr>
<tr>
<td>RGA-1 expression in control platelets, patient sera, after OT (ratio)</td>
<td>1.40 (1.19-1.60)</td>
<td>0.85 (0.61-1.24)</td>
</tr>
</tbody>
</table>

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 (fibrinogen). 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 treatment 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
BORTEOZIMB 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 delivery of the injection can take considerable time. From a patient perspective, significant amount of time spent without economic activity and travel costs can be considerable.

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%) vs 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 63 min (range: 5-433 min). 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). 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: Azacitidine is approved in the UK for the treatment of elderly patients with myelodysplastic syndromes (MDS) who are not suitable for allogeneic stem cell transplantation. This is an oral hypomethylating agent that is fully covered by the National Health Service (NHS) for use in the community. From a patient perspective, oral administration of azacitidine is associated with a reduction in healthcare related costs, time off work and access to specialist care. Its use in the community is associated with significant economic advantages. To date no studies have compared hospital vs. home treatment for azacitidine.

Aims: We sought to compare the costs of hospital vs. home treatment of azacitidine for patients with MDS. We also assessed patients preferences for hospital vs. home care.

Methods: A retrospective cohort study was conducted in the Limousin region of France. The regional hematological network (CRFF 23) provided patient characteristics and treatment details. The economic activity of patients and health care providers was estimated. The costs to patients of delivering azacitidine at home was estimated and compared to hospital delivered azacitidine. Data was collected for a 2 year period. Median age was 70 years-old (yo) (39-95); Male 74 patients (58%) vs 53 patients (42%).

Results: We identified 127 patients who incurred a total of 2,134 visits whilst receiving Azacitidine in the home setting. 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 Azacitidine: the median time from patient registration to azacitidine administration was 63 min (range: 5-433 min). Pharmacy cost for preparation of Azacitidine was £50 per dose. The cost of delivery of azacitidine (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). 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.
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 (CMML) with 10-29% mediulary 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 mediulary blasts. Azacitidine is drug administration and 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) that facilitates chemotherapy administration via local hospital at Home (HaH) establishments, which is an alternative to conventional hospitalization in France (www.ihehac.fr). The aim is to minimize the frequent hospital visits that these treatments require. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated treatments. This organization, including the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated treatments, requires. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated treatments. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated treatments. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated treatments.

Methods: Bortezomib, another injectable chemotherapy (Touati et al., 2013), was randomized and patient blinded trial aimed to evaluate, as primary end point, the efficacy and safety of opioid and benzodiazepine agent combination plus LIA in patients who underwent BMAB for hematological malignancies. Two secondary end points were: 1) define if patients who already underwent to BMAB without LIA prefer sedoanalgesia; 2) sedoanalgesia can influence the outcomes to minimize the frequent hospital visits that these treatments require. This organization includes the three previously mentioned hospitals, four HaH structures, and three central pharmacies with an integrated treatments.

Aims: Our work aimed to demonstrate that HaH administration of AZA is feasible and well preferred by patients compared to hospital administration.

Results: From 2009 to 2015, a total of 11.367 infusions were administered at home for 464 pts. In 2016, we demonstrated the feasibility of ESCADHEM (20% pts with AML/MDS were treated by AZA). The overall satisfaction rate was 95%.

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 last infusion (D1) of subsequent cycles were administered at the outpatient care unit. The following injections were administered at the patient’s home and carried out by HaH, according to a predefined procedures. Those patients had previously undergone this procedure without premedication, 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.

Figure 1.

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.

Table 1.

Summary/Conclusions: This important number of subcutaneous injections (n=6369) in a large cohort (n=169) over a period of time of 6 years activity show that home administration of subcutaneous AZA is feasible without serious adverse event. The first satisfaction survey demonstrates a high rate of satisfaction by all patients (20% with AML/MDS) treated by injectable chemotherapy at home. 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.

E1459

USE OF COMBINED ORAL FENTANYL CITRATE (ACTIO®) AND MIDAZOLAM AS PREMEDICATION FOR BONE MARROW BIOPSY IN PATIENTS WITH HEMATOLOGICAL MALIGNANCIES: A RANDOMIZED CONTROLLED PATIENT BLINDED CLINICAL TRIAL

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E1460

ASSESSMENT OF THE ECONOMIC IMPACT OF HORSE-ATG IN SWEDEN FOR APLASTIC ANAEMIA

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Background: Aplastic anaemia (AA) is a rare, potentially fatal haematopoietic stem-cell disorder that can either be inherited or acquired. AA is graded according to disease severity, from non-severe to very severe and is linked to immune-related responses such as the destruction of bone marrow. Cases of severe and very severe AA are considered to be a haematological emergency requiring urgent treatment. Extended hospitalisations and the cost of treatments and disease management are associated with the economic impact of AA.
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 anemia (sAA) patients in Sweden.

Methods: A semi-Markov state-transition cohort model was developed to estimate long-term (up to 5 years) clinical and economic outcomes for patients with AA receiving either ATGAM or r-ATG as first-line IST treatment. The following key assumptions were included in the model: responders who relapse are assumed to be re-treated with no expected change in survival. Patients who do not respond to first-line treatment move onto a second-line treatment comprised of either IST, IST + eltrombopag or hematopoietic stem cell transplantation (HSCT). Although response rates are lower, those who respond to second-line treatment are assumed to have the same outcomes as those who respond to 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 anemia.

E1462

A CLINICAL AUDIT OF NUTRITIONAL SCREENING AND SUPPORT OF HOSPITALIZED PATIENTS WITH HEMATOLOGIC DISEASES

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Background: Poor food intake is a common problem in patients with hematologic diseases. Recurrent infections and chemotherapy complications are some of the possible causes. Malnutrition is correlated to slow recovery, prolonged hospitalization, and higher mortality. Audits about the nutritional support of hospitalized patients may detect significant failures in patient care and help towards the correct application of the international guidelines.

Aims: We performed a prospective observational audit on hospitalized patients with hematologic diseases to investigate their nutritional status and whether they received the appropriate nutritional support.

Methods: The initial population consisted of 122 consecutive patients with hematologic diseases admitted from March 31, 2016 to June 8, 2016 in two Hematologic Units of a Tertiary University Hospital in Athens, Greece. We designed a special questionnaire based on the Malnutrition Universal Screening Tool (MUST) with additional questions on demographic, somatometric and medical data (Table 1). The questionnaire was applied by 6th-year medical students to all patients within 48 hours of admission. Patients were classified as high, intermediate, and low-risk per the MUST score and were reassessed at prede- fined intervals. We examined the food intake and the nutritional interventions (nutritional supplements, enteral or parenteral nutrition) applied.

Results: Ninety-three patients were included in the final analysis (5 refused to participate, 22 were excluded due to short-term hospitalization, 2 were absent during reassessment). Forty-one (38%) patients had a MUST score ≥2 (high risk) but none of them received nutritional supplements. One patient was supported with parenteral nutrition (Table 1).

Table 1:

<table>
<thead>
<tr>
<th>Number of patients, N (%)</th>
<th>Age (years), median (range)</th>
<th>Gender (M/F)</th>
<th>BMI (kg/m², median)</th>
<th>% of unplanned weight loss in past 6 months, median (range)</th>
<th>Disease, N (%)</th>
<th>% of patients receiving nutritional support, N (%)</th>
<th>Reported food intake (last 5 days), N (%)</th>
<th>Serum albumin levels on admission/discharge (g/dl, median range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>93 (100)</td>
<td>57.5 (17-87)</td>
<td>1.4</td>
<td>25.39 (15.95-40.64)</td>
<td>3.6 (0-23.3)</td>
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</table>

Summary/Conclusions: Our audit revealed a lack of nutritional support of the hospitalized patients. A meeting with the involved health professionals was organized and an oral presentation of the results and the possible causes (lack of sensitization of the staff, high regimen cost, shortness of staff) was performed. Proposals to change the current situation were made such as detection of high-risk patients upon admission and further assessment by a nutritional specialist. A brief MUST-based questionnaire was also proposed to be used for all patients upon admission. A re-audit was programmed and is already in progress.

E1461

NEUROPSYCHOLOGICAL ANALYSIS OF LONG-TERM CONSEQUENCES OF ANTINEOPLASTIC TREATMENT

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Background: Study of long-term consequences of conducted antineoplastic treatment is becoming on the front burner because of significant increase in survival rates of Acute Lymphoblastic Leukemia (ALL) and Hodgkin’s Lymphoma (HL) patients. Modern therapeutic protocols (cranial irradiation and chemotherapy) can negatively affect patients’ cognitive functions and consequently decrease patients quality of life. It is therefore necessary to carry out qualitative and quantitative analysis of neuropsychological impairments in patients conducted antineoplastic treatment.

Aims: The aim of the research to study neuropsychological status (memory and executive functions) of adult patients after antineoplastic therapy in childhood.

Methods: Patients in a present clinical remission (5-14 years remission duration), N=60 (ALL), N=50 (HL). Mean age-14 (ALL), 19 years. N=60 control group. Qualitative, quantitative neuropsychological scales developed in Luria’s approach methodology (tests for dynamic praxis and memory domains) and one-way ANOVA on ranks were used.

Results: The main neuropsychological impairments were found in auditory-verbal memory and auditory-motor coordination (memory narrowing, violations in selectivity, inertia in mental process, perseverations, contaminations, difficulties in praxis automatisation). 20% of patients didn’t recall all words (auditory-verbal memory tests) after four representations (3,3% in control group, p<0,005). 19% of patients failed or had severe difficulties in dynamic praxis tests (2% in control group, p=0,005). The results point at possible deficits in temporal and frontal parts of the brain (predominantly posterior frontal and mediobasal systems).

Summary/Conclusions: Neuropsychological impairments in audioverbal, auditory-motor and dynamic praxis spheres, qualitative and quantitative analysis showed deficits in temporal and posterior frontal areas of the left hemisphere, that can be as a result of antineoplastic therapy in childhood. Such findings can improve understanding the nature of patients impairments and difficulties they experience. Rehabilitation programs should take into account patients neuropsychological changes and cognitive decline. Indeed psychological interventions are necessary in long-term period after clinical remission.
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 fit-ness to safely undergo the procedure. More recently introduced tx options have significantly increased the life expectancy of pts with MM and continue to pro-vide further promise for the future in this devastating disease. The increasing therapeutic armoury across the MM pathway allows for varied tx patterns pro-viding 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 pro-vided data on consecutive pts with MM who had undergone an ASCT as part of 1L tx or on or after 1st January 2014, to specifically examine the HCRU post 1L ASCT. Data collected pertained to pt characteristics, b. patterns, duration of tx and outcomes (including time to progression (TTP) and best response achieved (IMWG updated criteria), TTP 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 diag-nosis was 59 (±7.8 SD) years; 43% female and 57% male. Mean duration from diagnosis, to receiving an ASCT was 9.6 months (±13.3 SD). Of the pts included in the study, 62%, 28% and 8% had received 1st, 2nd and 3rd line tx respectively. In the 1L setting, 72% of pts did not receive any drug therapy post ASCT, 21% received consolidation and 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 (10%). Of 1L pts, TTP from start of 1L tx was 22.2 months (±11.1 SD) for pts not receiving maintenance and 33.0 months (±8.1 SD) for pts receiv-ing maintenance. Overall 43% of pts achieved a sCR and CR, 51% achieved a VGPR and PR. The period during which 1L pts post ASCT to start of 2L, 54% of pts required supportive tx (bisphosphonate (55%), blood transfusions (24%), G-CSF (21%), ESAs (11%), radiotherapy (4%) and dialysis (3%)). 64% of pts were hospitalized at least once during this period, with a mean duration of 7.2 days (±18.1 SD). The mean number of visits to Hematologists was 7.1 times in 24.8 months (between start of 1L to start of 2L b); mean visits to a HCP dur-ing this period were 17. The mean TTP from start of 2L tx was 11.2 months (±6.8 SD); 20% of pts achieved a sCR and CR, 52% achieved a VGPR and PR.

Summary/Conclusions: The sample is reflective of the pt demographics data reported in Raab et al. 2016. Furthermore, the TTP for pts not receiving any active ongoing tx post ASCT in this real-world study is comparable to findings in active ongoing tx data exists on HCRU post ASCT. This study demonstrates that there is ongoing HCRU impact even if pts are not receiving any active ongoing tx post first ASCT. Prolonging the remission period post ASCT may therefore spread the marginal cost of HCRU whilst simultaneously enhancing a pt’s quality of life by deferring future tx lines.

E1465

THE COST-EFFECTIVENESS OF PEGASPARAGASE FOR FIRST-LINE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKAEMIA: A COST-UTILITY ANALYSIS

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Background: Asparaginase is a key component in the multi-agent chemotherapy regimen for the treatment of children, adolescents, and adults with acute lymphoblastic leukemia (ALL). Compared to native asparaginase (native ASP), pegaspargase (PEG-ASP) has a longer half-life, can be given less frequently, and is less immunogenic, which leads to fewer hypersensitivity reac-tions. In the UK, patients with newly diagnosed ALL are treated with PEG-ASP followed by Erwinia-derived asparaginase (ERW-ASP) in cases of hypersen-sitivity, 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 evalu-ate overall cost-effectiveness of PEG-ASP in comparison to native ASP when utilized as part of antineoplastic combination therapy for treating newly diag-nosed 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 mod-elled. 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 1,000IU/m2 dosing (per UKALL protocols) used for all regimens. The 2,000 IU/m2 dosing of native ASP was examined, as well as variations in comparative survival and hypersensitivity rates. Incremental cost-effectiveness ratios (ICER; defined as incremental costs/quality-adjusted life years [QALYs] gained) were produced.

Table 1.

<table>
<thead>
<tr>
<th>Technologies</th>
<th>Total</th>
<th>Incremental</th>
<th>ICER (£)</th>
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<tbody>
<tr>
<td>PEG-ASP &gt; ERW-ASP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NNT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-month cost</td>
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<tr>
<td>4.5 (3.4-6.7)</td>
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<td>$164,000 ($124,000-$240,000)</td>
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<tr>
<td>$161,000</td>
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<tr>
<td>$141,000 ($75,300-$377,000)</td>
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<td>$130,000 ($106,000-$161,000)</td>
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<tr>
<td>$141,000 ($75,300-$377,000)</td>
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<td>$164,000 ($124,000-$240,000)</td>
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* Dominated indicates more costly and less efficacious compared to the base-case model.
Results: The base-case scenario demonstrated that PEG-ASP followed by ERW-ASP dominated (i.e., was 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-0199) MONOTHERAPY
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Background: Chronic lymphocytic leukemia (CLL) is associated with reduced health-related quality of life (HRQoL), with progressive severe fatigue being a particularly relevant burden. Disease-related symptoms, toxic effects of therapy, and the awareness of living with an incurable disease can have a profound impact on HRQoL. Venetoclax (VEN) is an oral BCL-2 inhibitor that has demonstrated deep and durable responses in relapsed/refractory (R/R) CLL patients.

Aims: To assess whether Venetoclax has a sustained impact on health related quality of life among patients with relapsed/refractory CLL based on a second interim analysis (first interim results through week 24) of patients treated with VEN monotherapy.

Methods: Patients ≥18 years of age with R/R CLL received VEN monotherapy until disease progression, unacceptable side effects, or discontinuation for any other reason. Patient-reported HRQoL measures included the EORTC QLQ-C30 and EORTC QLQ-CLL16 from Baseline (BL) to each assessment after 4 weeks and every 12 weeks thereafter. Changes from BL to each assessment are reported. Clinical relevance was based on minimum important difference (MID) of values from BL at different assessment points. The lower bound of 5–10 point changes, considered a ‘little’ change for EORTC QLQ-C30 and a 10% change for EORTC QLQ-CLL16, was used as MID acceptance for both measures.

Results: Clinically meaningful improvements from BL were observed early and were sustained throughout weeks 96 in VEN treated patients in the EORTC-QLG-C30 global health status and the role, social, and emotional functioning scales. Improvements in VEN treated patients in EORTC-QLG-CLL16 disease effects, social problems, and future health worries scores were statistically significant and exceeded the MID at all assessment points. Furthermore, early and sustained improvements in fatigue through week 96 were seen in both EORTC-QLG-C30 and EORTC-QLG-CLL16 (Table 1). The changes observed in patient EORTC-QLG-CLL16 future health views were considered large (>20 points) at Weeks 12, 24, and 48.

Table 1.

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 HAEMATOLOGICAL CONDITIONS CAN THIRD YEAR MEDICAL STUDENTS RECOGNIZE INTERPRETING FULL BLOOD COUNT RESULTS?
S. Lovato1,2, J. Arnold1,2

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.

E1468
LONGITUDINAL ASSOCIATIONS BETWEEN HEALTH-RELATED QUALITY OF LIFE AND HEALTHCARE UTILIZATION IN AL AMYLOIDOSIS
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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.
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., out-of-pocket costs for healthcare visits, laboratory tests, drugs and hospital stays) 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 healthcare visits or with 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 around 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 in patients with chronic diseases.

Aims: This prospective, randomized and controlled study tested the safety, efficacy and feasibility of EMS in 72 patients (EMS=42, control=30) undergoing autologous HSCT (n=21), allogeneic HSCT (n=17) and intensive chemotherapy (n=34).

Methods: A Myopuls 2000 device (Curatec Services GmbH) was used. Target training time was 15 minutes five days a week on both thighs and arms from start of therapy (T1) to time of discharge (T2). Adverse events and treatment adherence were documented. Impact on psychological and physical functioning was evaluated using the Multidimensional Fatigue Inventory (MFI), the EORTC QLQ-C30, the Short Physical Performance Battery and the 6 Minute Walking Distance test at T1 and T2.

Results: Seven patients died in the EMS- (n=4) and control-group (n=3). 6 of 42 EMS patients withdrew because of sepsis (n=4) or loss of motivation (n=2). 32 patients from the EMS group completed our study with 22 accomplishing >66% of the pre-set training time. EMS related adverse events were headache (n=1) and muscle pain (n=2). No bleeding events (WHO bleeding scale 1) or ventricular arrhythmias occurred. Difference in 6-minute walking distance between both groups was 23 meter (p=0.2). SPPB test results differed by one point (p=0.08). MFI and EORTC QLQ-C30 both favoured the EMS group, but showed no statistical significance.

Summary/Conclusions: EMS is feasible and safe in patients undergoing intensive chemotherapy regimens. It also may improve physical fitness, fatigue and quality of life, indicated by favourable test results in the EMS group. To verify positive effects of EMS in patients with haematological malignancies, further research is needed, with more patients and sham EMS stimulation.

E1470
MEYLOMA 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 adverse effects. Therefore, myeloma patients face difficult decisions about the benefits and risks of treatment. The purpose of this study was to assess myeloma patient preferences for treatment.

Aims: The study aimed to answer the following questions: What treatment attributes do myeloma patients value? What is the relative importance of different treatment attributes and the maximum acceptable risk they are willing to accept? What risk-benefit trade-offs characterise patients’ decision-making around treatment options, including not to treat? What, if any, influences and predictive factors are found in the way patients assess benefits and risk?

Methods: Participants were 475 Myeloma patients in the UK. Data were collected using discrete choice experiments (DCEs) through an online survey. The DCEs presented patients with a traditional treatment choice experiment (e.g., treatment A vs treatment B), focusing on the clinical benefits of treatments and the associated risks. The attributes and levels of the attributes were selected based on previous research, literature review and expert opinion. The DCE data were modelled using a Latent Class Model (LCM), and the effect of demographic characteristics were also examined.

Results: Findings revealed two classes (groups) of patients with different preferences for attributes. 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 average out-of-pocket costs.
**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 myelogogenous leukemia (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 leukemia. We have established QoL scores given by the questionnaire. FACIT, consisting of three levels: TOI for leukemia trial outcome index, FACT-G for general score, and FACT-LEU for the total score of leukemia. 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 malignancies and its goal include patients achieve levels of quality of life (QOL) similar that general population. However, studies developed in Europe and United States have shown that patients on long-term follow-up after HSCT reported lower levels of QOL, more unemployment and lower household income than before the procedure. These relationships have not been examined in Mexican HSCT patients.

**Table 1.**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Median (range)</th>
<th>Percentages of participants with low QoL</th>
<th>Bivariate Chi-Square test (UH vs. MH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global QL</td>
<td>79.4 (20 – 100)</td>
<td>79.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Physical QL</td>
<td>90.8 (20 – 100)</td>
<td>90.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Role QL</td>
<td>65.9 (20 – 100)</td>
<td>65.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Cognitive QL</td>
<td>66.7 (20 – 100)</td>
<td>66.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Emotional QL</td>
<td>66.7 (20 – 100)</td>
<td>66.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

**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 25-56), 56% male, and 41% married. Regarding educational level 68.7% had basic education, 25% had a college education and 6.3% postgraduate education. Mean time after HSCT was 36 months, 10% had active chronic graft versus host disease (GVHD). Patients reported moderate to high levels of QOL (Table 1). With respect to employment, 52% had a job (56% had a full time job, 13% worked part-time and 31% had an informal job) and 48% were unemployed (50% could not find a job and 50% did not want to have a job). Finally, 56% had lower household income than before HSCT.

**Summary/Conclusions:** Mexican patients showed similar or higher levels of QOL in comparison with samples from other countries, with the exception of higher impact in emotional QOL and better social QL in our sample. Additionally, a substantial minority of patients were unemployed and over half had lower household income after HSCT. More work is needed to identify risks associated with changes in QOL, employment status and income among long-term survivors of HSCT.

**E1474**

ANTHRACYCLINE INCREASES THE RISK OF DEVELOPING DIABETES IN B CELL LYMPHOMA

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**Background:** Treatments of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) or R-CHOP like regimens have made B cell lymphoma to be one of the most curative hematological malignancies. Among the effective chemotherapy 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 3984 B cell patients were analyzed. To understand whether anthracycline therapy was associated with more diabetes in B cell lymphoma, we compared the cumulative incidence of newly diagnosed diabetes between patients with (n=3147) and without (n=837) anthracycline treatments. Impact of anthracycline on diabetes was further studied by multivariate Cox proportional hazard regressions in a dose-dependent manner.

**Results:** Log-rank test did not show the difference of cumulative incidences of newly diagnosed diabetes between B cell lymphoma patients with and without anthracycline treatments (p=0.1448). However, anthracycline remained associated with more diabetes [hazard ratio (HR): 1.59; 95% confidence interval (CI): 1.05–2.39; p=0.0278] after adjustment for age, gender, and comorbidities. Moreover, cumulative anthracycline doses of 253-400mg (HR: 1.94; 95% CI: 1.23–3.05; p=0.0043) and 401-504mg (HR: 1.83; 95% CI: 1.11–3.01; p=0.0180) increased the incidence density of diabetes in a dose-dependent manner (p=0.0438). Notably, patients with and without anthracyline treatment had similar yearly adapted diabetes complications severity index alteration (0.58±1.89 vs 0.75±1.85; mean±standard deviation), suggesting anthracyline did not deteriorate outcome of diabetes in B cell lymphoma patients (p=0.4924).

**Figure 1.**

Madrid, Spain, June 22 – 25, 2017
Summary/Conclusions: Anthracycline therapy was responsible for more diabetes and cell lymphoma in a dose-dependent manner. More intensive blood sugar monitoring and control should be recommended to B cell lymphoma patients, especially those who received anthracycline treatment.

E1475
THE COST-EFFECTIVENESS OF LENALIDOMIDE PLUS DEXAMETHASONE FOR THE TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA IN CHINA
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Background: The introduction of lenalidomide plus dexamethasone (RD), and bortezomib-containing regimens, has improved the management of relapsed or refractory multiple myeloma (rMM) in China. However due to the absence of both head-to-head (direct) comparative efficacy and local economic data, stakeholders still face hard choices to make when choosing one therapy over another. Indirect treatment comparisons and health economic modeling can help support decision-making by enabling the incorporation of country-specific unit cost data, in the comparison of cost-effectiveness of one treatment vs another where treatments have not been directly compared in clinical trials.

Aims: To assess the cost-effectiveness of RD relative to bortezomib/dexamethasone (VD) and bortezomib/cyclophosphamide/dexamethasone (VCD) for rMM in Chinese patients.

Methods: The Markov-based decision analytic model was constructed to simulate lifetime health benefits and direct medical costs associated with RD, VD, and VCD for rMM in Chinese patients. A systematic literature review was conducted (in both Chinese and English databases, from 2005 to 2016) to obtain efficacy data of the three treatment regimens. The risk of progressive disease associated with RD and VD were estimated from available Chinese trials. The efficacy of VCD and the mortality associated with progressive disease after treatments with RD and VD were lacking in China, therefore were estimated from the published international randomized clinical trials. Published quality of life data was adapted to Chinese rMM patients with health utility adjustment. The model took into account (i) drug acquisition costs, (ii) treatment administration costs of Chinese urban hospitals, (iii) serious adverse events management costs based on a survey of seven MM centers across China, and (iv) rMM management costs estimated from a Chinese real-world hospital setting. Quality-adjusted life years (QALY) and direct medical costs in the model were discounted at 3% per annum. Base case analysis calculated incremental cost-effectiveness ratio (ICER) 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 international 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 (2.37 vs 0.78) and VCD (2.37 vs 1.36). RD was associated with longer disease-free survival (1.41 vs 1.11) and lower health care acquisition costs ($49,060 vs $272,135 vs $448,220) than both VD and VCD. The ICERs of QALY for RD relative to VD ($194,706) and VCD ($150,774) were less than the cost-effectiveness threshold of China (three times of estimated 2016 China GDP per capital $166,920/QALY, $1= €0.138). The cost-effectiveness of RD was robust to a range of ICERs per QALY for RD relative to VD, VCD 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 international randomized trials for the efficacy associated with RD, VD, and VCD, to verify the base case analysis.

Summary/Conclusions: The findings of the content validation, pretesting and cognitive interviews indicate that HM-PRO possesses a strong content validity in different HMs, includes all the issues important to these patients and the statements are easy to read, understand and respond to spontaneously. HM-PRO will undergo further psychometric testing to support its psychometric properties across different types of HMs.

E1477
OVARIAN TISSUE CRYOPRESERVATION IN PEDIATRIC AND ADOLESCENT PATIENTS UNDERGOING CANCER CHEMOTHERAPY AND/OR HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background: Ovarian tissue cryopreservation (OTC) and subsequent reimplantation is the only option available for fertility preservation in prepubertal females, but this approach remains unestablished in pediatric and adolescent patients with cancer. After the experience of OTC for more than 200 patients with primary ovarian failure and more than 50 patients with breast cancer in our center over 5 years, we have started OTC for pediatric and adolescent cancer patients since 2015. Aim: To evaluate safety and benefits of OTC in pediatric and adolescent patients 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 primary ovarian failure. 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. Result: Of the 5 ovarian tissue samples collected, 6 were included in the study 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 ovariun function and fertility preservation in pediatric and adolescent cancer patients. A risk of reseeding malignant cells is a problem still to be conquered.

E1479

A MULTIDISCIPLINARY APPROACH TO CHEMOTHERAPY PRESCRIBING AT NEWCASTLE UPON TYNE HOSPITALS NHS FOUNDATION TRUST S. Gabriel1,*, G. Jones2, S. Blakey2, M. Lannon2

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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 supplemental medications are approximately 30 minutes.

Aims: We introduced a weekly multi-disciplinary chemotherapy prescribing meeting in 2013 with the aims of improving prescribing safety, minimising time spent prescribing in clinic & reducing patient waiting times. Present at each meeting is a Haematology Specialist Pharmacist, Haematology CNS, Consultant & Specialist Registrar. Chemotherapy is planned a week in advance on ChemCare (an electronic chemotherapy prescribing package). Chemotherapy is prescribed in the clinic & 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 line with 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, 68 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 incurred in the old system. However, it must be noted that during Jan-June 2015 the cost of wasted drug, due to patients being unfit for treatment on the day, potentially incurred by pre-prescribing was £57,775. It is, therefore, critical to ensure that medication that has not yet been given to patients can be returned to pharmacy if this type of pre-prescribing model is to operate efficiently.

E1479

FINANCIAL TOXICITY OF THE MANAGEMENT OF MULTIPLE MYELOMA B. Sidi Mohamed El Amine1,*, H. Asma1, O. Fouzia1, S.A. Najet1, Z. Zahia1

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Background: Advances in supportive care and the development of novel treatment therapies 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 complete the survey before their visit or drug administration. 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.

E1480

THE IMPLICATIONS OF NON-PROPORTIONAL HAZARDS FOR THE MEASUREMENT OF SURVIVAL BENEFIT IN HEALTH TECHNOLOGY ASSESSMENT: CURRENT APPROACHES AND THE ROLE OF RESTRICTED MEAN SURVIVAL TIME G. Monnickendam1,*, J. McKendrick1, M. Zhu1, Y. Su2

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Background: Median survival and hazard ratios (HR) calculated from Cox proportional hazard (PH) models for progression-free survival (PFS) and overall survival (OS) are principal survival endpoints in oncology trials. The advent of novel agents, including immuno-oncologics, has seen increasing reports of non-proportional hazards (non-PH). When non-PH are present, it is challenging to evaluate the true clinical significance of survival differences even if median and mean survival times do not represent the comparative benefit over the full period of observed data. In such situations, additional metrics such as restricted mean survival time (RMST) may be valuable.

Aims: To determine current methods used by health technology assessment (HTA) agencies when non-PH are observed in assessments of hematological/oncology drugs, and to explore if RMST is used as an alternative measure of treatment benefit in these circumstances.

Methods: Methodological guidelines published by 10 HTA agencies in 8 major developed countries (Australia, Canada, France, Germany, Italy [Emilia Romagna, Veneto], Spain, Sweden, and the United Kingdom [NICE, SMC]) and by international organizations (including the US (including the Institute of Clinical and Economic Review, ICER) were reviewed to establish recommended approaches for presenting survival benefit from clinical trials, particularly the use of RMST where non-PH were reported. To determine how these guidelines are implemented in practice, published HTA reports were examined across the 8 countries for 23 oncology agents (including 4 in hematology) approved by the FDA and EMA since 2014, to identify instances where testing for non-PH was conducted and RMST data reported.

Results: Guidelines from only 2 agencies (PBAC in Australia and NICE in the UK) described formal testing for non-PH. Testing was reported in 4 (of 6) NICE assessments and 4 (of 10) PBAC assessments. For the hematology drugs, non-PH testing was conducted in 3 (of 4) NICE assessments; it did not hold in 2 instances. Of the agencies (from France, Germany, Italy and Spain, which focus on comparative clinical benefit, only 1 (GENESIS in Spain) discussed the concept of RMST in their guidelines; 5 (of 45) assessments from these countries included non-PH. Of the agencies (from Australia, Canada, Sweden, and the UK) which focus on cost-effectiveness, all except the TLYV in Sweden include RMST in their guidelines; RMST was reported in 13 (of 81) HTA assessments from those countries. Of the 3 hematology drugs where non-PH was tested within the NICE peripheral neuropathy trials (of which there were 4 reported RMST [utilized during economic model sensitivity analyses]. Non-PH is not a widely reported issue in US guidelines; however, the ICER has acknowledged it and PH testing was conducted in both ICER reports in oncology.

Discussion: Testing for non-PH is not widely reported in clinical trials or incorporated into assessments by HTA agencies except by UK NICE. RMST as a metric to assess OS has played a role in assessing clinical benefit within the context of HTA assessments, although not consistently within countries (across trials) or across countries (for the same drug), as was seen with the hematology agents. As treatments for cancer expand to new classes and indications, instances of non-PH will likely increase; alternative survival metrics such as RMST may have an increasingly important role to play in describing survival benefit in such cases.
Sickle cell disease

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 β-thalassemia [HbSSβ-]) or “moderate” groups (compound heterozygous for HbS, with either HbC [HbSC], or β-thalassemia [HbSββ-]). Standardized DSST scores based on published norms were used to define mild cognitive impairment, defined as ≤1.5 standard deviations (SD) below the DSST T-score (T-scores had a mean of 50 and SD of 10).

Data on demographics, hematological parameters, hydroxyurea and opiate intake, stroke (including silent cerebral infarcts [SCI]) and transfusion history were collected concurrently with DSST. Analyses were repeated after exclusion of patients with a history of stroke (n=12). Age-adjusted p-value was calculated with logistic regression for all variables except age (unadjusted) and DSST T-score (already adjusted for age, sex and education in calculation of T-score).

Results: Among our patients, 56 (63%) had a “severe” genotype and 32 (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>Variable</th>
<th>&quot;Severe&quot;</th>
<th>&quot;Moderate&quot;</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.7 (10.8)</td>
<td>40.9 (12.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>Male sex*</td>
<td>21 (37.5%)</td>
<td>12 (37.5%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.1 (1.8)</td>
<td>13.2 (1.7)</td>
<td>0.80</td>
</tr>
<tr>
<td>Mild Cognitive Impairment*</td>
<td>17 (30.4%)</td>
<td>3 (9.4%)</td>
<td>0.14</td>
</tr>
<tr>
<td>DSST T-score</td>
<td>47.6 (14.5)</td>
<td>51.0 (13.4)</td>
<td>0.006</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 (X 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)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Platelet count (X 10^9/L)</td>
<td>344.1 (179.8)</td>
<td>263.3 (115.1)</td>
<td>0.16</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.7 (4.0)</td>
<td>1.1 (1.8)</td>
<td>0.51</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>32.1 (14.3)</td>
<td>269.2 (14.9)</td>
<td>0.18</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>114.1 (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>
</tr>
<tr>
<td>SBP (mm/Hg)</td>
<td>111.3 (13.4)</td>
<td>118.9 (13.6)</td>
<td>0.08</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>68.8 (7.7)</td>
<td>73.5 (18.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>MAP (mm/Hg)</td>
<td>83.1 (8.4)</td>
<td>88.6 (10.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Hydroxyurea use*</td>
<td>32 (67.1%)</td>
<td>10 (31.2%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Opiate use*</td>
<td>15 (26.8%)</td>
<td>10 (31.2%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Transfusion history*</td>
<td>17 (31.5%)</td>
<td>5 (16.1%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Stroke history‡</td>
<td>10 (18.2%)</td>
<td>2 (6.2%)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Mean (SD) unless otherwise noted. † Age-adjusted. P1 includes SCD.
Summary/Conclusions: Early identification of chronic hepatic disease sometimes pauci-symptomatic in terms of VOCs but able to lead to advanced stage and progressive fibrosis is crucial for suitable clinical management to avoid cirrhosis in SCD patients. The combination of TE with specific serum markers (GGT, ALP, albumin) is a valid tool to early detection of sickle hepatopathy.

E4183

MICROSTRUCTURAL ANALYSIS OF RETINO-CHOROID LAYERS USING OPTICAL COHERENCE TOMOGRAPHY IN ADULT PATIENTS WITH SICKLE CELL DISEASE

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Background: Retinopathy is one of the ophthalmological complications of patients with Sickle cell disease (SCD), due to microvascular occlusions; occasionally, proliferative sickle cell retinopathy (PSR) can lead to severe vision loss. Aims: a. to analyze macular alterations in patients with Sickle Cell Disease (SCD) by spectral-domain optical coherence tomography (SD-OCT), using the automated software for retinal segmentation; b. to investigate relationship between OCT abnormalities and the severity of proliferative sickle cell retinopathy (PSR); c. to elucidate the role of potentially contributory systemic factors on the development of macular thinning.

Methods: This is a prospective, observational case-control study. Ophthalmological evaluation, fluorescein angiography and SD-OCT were performed. Central and temporal retinal layers were measured by the SD-OCT Automatic Segmentation software. SD 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±6.89 (M.F 12:18), including 9 patients with Sickle Cell Anemia (SCA), 17 with Sickle Cell A-Thalassemia and 4 Sickle Cell B-Thalassemia and 4 Hbs/Hbc. One HbsB/Hbc was excluded 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 SD-OCT, mostly seen temporally to the fovea. Among these patients, 6 had SCA, 4 had B-thalassemia, and 4 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). SD eyes with patchy retinal thinning showed significant inferior inner nuclear layer (INL) and outer plexiform layer (OPL) in the temporal region. Univariate analysis revealed a significant correlation between patchy areas of severe retinal thinning on OCT and SCD need for transfusions, need for chelation, Hbf, ferritin, and transferrin saturation (p<0.001). Logistic regression model showed that most predictive variables for retinal thinning as assessed after multivariate regression analysis were the need of chelation (p=0.0187) and the Hbf (p=0.0775). More specifically, the odds of retinal thinning is 94.2% lower when chelation is present, and the odds of retinal thinning decreases by 12.9% when Hbf increases by 1 unit.

Summary/Conclusions: In this study SD eyes of all patients showed both inner and outer retinal thinning in the central and temporal macula. Ischemia caused by chronic occlusion of the deep and superficial capillary plexus could explain the different retinal layers’ damage and the pattern of thinning. No major statistical differences were found between the three sickle genotypes because of small number of patients we have. Retinal thinning impacts visual function and can lead to irreversible visual loss, regular ocular checkups are essential for SCD patients.

E4184

NON ABLATIVE TRANSPLANT CONDITIONING WITH TREOSSULFAN IS CURATIVE IN A 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, hyposthanuria 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 (Treoosulfan).

Aims: Optimize non-myeloablative conditioning in a murine model of SCD that allows for sufficient donor RBC chimerism.

Methods: Control (AA) and SCD (SS) animals were treated with varying conditioning regimens (+/- rescue with AA or SS marrow), including Trewosulfan (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, reticuloocytes, 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 moribund animals underwent necropsy to assess organ damage.

Results: Erythroid hyperplasia was noted in the BM of SS, relative to AA mice. Trewosulfan, in a dose-dependent manner, decreased BM cellularity and induced cytopeinia in AA and SS mice. AA mice were able to tolerate Trewosulfan 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 chimeraism at one year. In SS mice achieving 50% AA in peripheral blood, fertility was preserved, and increased survival. SCD pathology was not comparable to age-matched controls. ACK2, anti-CD40L, or low-dose radiation, in combination with Trewosulfan (3g/kg), failed to improve engraftment. In contrast, increasing Trewosulfan 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 haptoglobin. 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. Trewosulfan, at sub-myeloablative dosing, sustained erythroid chimerism and reversed the SCD phenotype. Our data suggests that pre-transplant conditioning with Trewosulfan alone may be permissive for engraftment, in an allogeneic and gene-corrected autologous clinical transplant setting.
4/61 (1.6%), positive D-Dimers in 57/59 (96.6%), decreased protein S in 10/61 (16.3%) and decreased protein C or 13/61 (21.3%), NATEM MCF was increased in 27/61 (44.3%) patients while EXTEM MCF was increased in 31/61 (50.8%) patients. Patients with a history of TEE had higher mean values of NATEM-MCF and EXTEM-MCF and those differences were statistically significant (p=0.023, and p=0.011 respectively). There was a statistically significant association between the presence of ischemic lesions in brain MRI and the history of TEE (p=0.01). On the contrary, the history of ACS was not correlated with the presence of ischemic lesions in MRI. Chronic Hydroxyurea treatment did not correlate with the absence of ischemic findings in brain MRI. Among patients with ischemic lesions those who were already on chronic hydroxyurea treatment had a shorter NATEM-CT compared to patients without treatment. In patients with ischemic lesions in MRI and a history of TEE NATEM-MCF and EXTEM MCF were higher (p=0.03, and p=0.03, respectively).

Summary/Conclusions: The presence of microschemic encephalopathy is very common in SCD patients and is associated with a history of TEE, which is also frequent in SCD. There seems to be a permanent activation of the coagulation mechanism in SCD. In SCD patients with SCIs and a history of TEE, apart from clotting factors and natural inhibitors there seems to be a contribution of platelets and cellular elements, possibly sickle cells. The impact of chronic hydroxyurea treatment on the pathogenesis of silent infarcts and TEEs needs further evaluation.

E1486
Abstract withdrawn.

E1487
INVASIVE BACTERIAL INFECTIONS IN GAMBIAN PATIENTS WITH SICKLE CELL ANEMIA IN AN ERA OF WIDESPREAD PNEUMOCOCCAL AND HAEMOPHILUS INFLUENZA TYPE B VACCINATION
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Background: Bacterial infections cause significant morbidity and mortality in patients with sickle cell anemia, especially in populations without reliable access to antimicrobial prophylaxis and treatment. The last and astounding use of penicillin prophylaxis and vaccination for Streptococcus pneumoniae and Haemophilus influenzae type b in resource-rich settings has minimised the additional risk of invasive bacterial infections associated with sickle cell anemia. However, these interventions are not routinely implemented in much of Africa, despite this region having the greatest burden of disease, with over 80% of people with sickle cell anemia born on the continent. The Gambia has well established vaccination programmes for pneumococcal and Haemophilus influenzae type b, which is rare in the region. There is little data on the incidence of bacterial infections in African sickle cell anemia populations, and we believe (until this study) there were no data from countries with comprehensive vaccination programmes against Streptococcus pneumoniae and Haemophilus influenzae type b.

Aims: Primary: to determine the predominant pathogens causing invasive bacterial infections in a population of sickle cell anemia patients admitted to the Medical Research Council Unit Gambia. Secondary: to review the characteristics of the sickle cell anemia population.

Methods: A retrospective analysis of the clinical and laboratory records relating to 161 admissions of 126 patients with sickle cell anemia admitted to the Medical Research Council Unit Gambia over a five-year period (between April 2010 and April 2015) when there was high coverage of pneumococcal and Haemophilus influenzae type b vaccination.

Results: Pathogenic bacteria were cultured from blood in 11 of the 131 admissions which had blood cultures taken (8.4%, 95% CI 4.5-14.1%). The most frequent organism isolated was Salmonella typhimurium (6/11; 54.5%), followed by Staphylococcus aureus (2/11; 18.2%) and other enteric Gram-negative pathogens (2/11; 18.2%) and there was one case of Haemophilus influenzae non-type b bacteremia (1/11; 9.1%). No cases of bacteremia caused by Streptococcus pneumoniae or Haemophilus influenzae type b were identified. The most common diagnosis causing the admission was vaso-occlusive crisis (53/161; 32.9%), followed by infective complications including pneumonia (16/161; 9.9%) and osteomyelitis (12/161; 7.5%). The median length of admission was five days and the median age of patients was five years (IQR: 2-13 years). A new diagnosis of sickle cell anemia was made during the admission in just under half of patients.
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:

E1489
UNIVERSAL NEWBORN SCREENING FOR SICKLE CELL DISEASE: PRELIMINARY RESULTS OF THE FIRST YEAR OF A MULTICENTRIC ITALIAN PILOT PROJECT

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Background: Sickle cell disease (SCD) is the most common monogenic disease worldwide. Although it is most prevalent in Africa, in parts of the eastern Mediterranean and the Middle East region, the result of this disease varies from prevalence and is continuously increasing in central and northern Europe. It is established that early detection and appropriate prophylactic measures prevent potentially fatal complications and many European countries have already introduced newborn screening programs for SCD. In Italy it is estimated that 6.5% of the total population is represented by carriers of hemoglobinopathies, nevertheless, there isn’t a national newborn screening program for SCD nor a plan to establish it. Selective newborn screening programs for SCD are currently active in three regions of Italy, and a pilot universal newborn was terminated due to lack of funding. The aim of this study was to assess the feasibility and acceptability of a universal newborn screening program for SCD in Padova and in Monza.

Methods:
- Guthrie cards collected after parents informed consent, from an selected cohort of newborns, are analyzed by HPLC (Variant Newborn Screening-NBS-BioRad). The abnormal hemoglobin fractions identified by HPLC are confirmed by molecular analysis of the β-globin gene (HBB) by PCR and sequencing of the DNA extracted from the dried blood spot. Genetic counseling is offered to the families of infants carriers of the S gene, after notification of the results; families of all infants with SCD are contacted within two months for enrollment in comprehensive care.
- Results: Total of 9 months and 5 months 1364 and dried blood spots were collected in Padova and Monza respectively. Two families in Padova and 19 in Monza refused the test. The ethnic origin of newborns was similar in Padova and Monza. And 69.45% were of Italian couples, 9.29% of mixed couples and 21.26% of foreign couples in Monza. None of the couples live in the more rural PeeDee region and seek acute care from community hospitals. As a result, many of these patients have to travel >90 minutes for routine SCD care. Due to the difficulty in travel, many patients from this region were seen at frequent with key SCD clinics 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.
- Background: The primary aim of this study was to establish 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 clinical 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.

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 participating. An additional clinic was designated to serve pediatric patients with SCD and to assess the feasibility and acceptability of a telehealth clinic for the pediatric group for the telehealth clinic who had been designated as “lost to follow up (LTFU).” The clinics were originally scheduled monthly however three clinics were cancelled during the first 16 months and a total of 13 clinics were conducted. There were 64 total visits scheduled of which 50 visits were completed. The overall no-show rate was 14% (range 0-34%) and six clinics had a no-show rate of 0%. The scheduling rate was 78% (range 60-100%). The primary aim was to assess the feasibility and acceptability of a telehealth clinic measured by patients and families’ adherence to scheduled appointments. Of the original cohort of 19 patients (19) have continued participating in clinic (defined as attending more than 3 clinics in the 16-month period). Prior to starting 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 has continued monthly operations. Hydroxyurea initiation has improved and has been well tolerated by patients and families. Challenges have included equipment issues, difficulties in post-clinic care coordination and ensuring caregivers received discharge information. Future directions include a tele-telehealth clinic for 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 also harness the resources of the state to approach SCD and will also use a technology-based approach to increase education of providers.

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 to travel >90 minutes for routine SCD care. Due to the difficulty in travel, many patients from this region were seen at frequent with key SCD clinics 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.

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 spewal palpation techniques which cannot be performed using the telemedicine 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 central academic center (MUSC) living in this region were contacted to assess their interest in coming to the telehealth clinic.

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 participating. An additional clinic was designated to serve pediatric patients with SCD and to assess the feasibility and acceptability of a telehealth clinic for the pediatric group for the telehealth clinic who had been designated as “lost to follow up (LTFU).” The clinics were originally scheduled monthly however three clinics were cancelled during the first 16 months and a total of 13 clinics were conducted. There were 64 total visits scheduled of which 50 visits were completed. The overall no-show rate was 14% (range 0-34%) and six clinics had a no-show rate of 0%. The scheduling rate was 78% (range 60-100%). The primary aim was to assess the feasibility and acceptability of a telehealth clinic measured by patients’ and families’ adherence to scheduled appointments. Of the original cohort of 19 patients (19) have continued participating in clinic (defined as attending more than 3 clinics in the 16-month period). Prior to starting 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 has continued monthly operations. Hydroxyurea initiation has improved and has been well tolerated by patients and families. Challenges have included equipment issues, difficulties in post-clinic care coordination and ensuring caregivers received discharge information. Future directions include a tele-transectional 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 also 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.

**Background:** The incidence of the Sickle Cell Disease (SCD) has increased in Europe because of the high rate of migration from areas in which carriers of the sickle cell allele account for 19-27% of the entire population. Although SCD is endemic in Southern Italy, the recent migration fluxes spread SCD all over Italy with the number of carriers at about 6.5% of the whole population. The distribution of SCD patients has dramatically changed. The large part of resident immigrants are young with an high fertility rate. Neonatal screening combined with timely diagnostic testing, parental education and comprehensive care management has reduced morbidity and mortality of SCD. Up to now, a national newborn screening program for SCD is not active in Italy and only few pilot studies have been carried out (Ballardini E et al. Blood Transfus. 2013 Apr; 11(2): 245-9; Venturelli D et al., Blood Transfusion 2014; 12: 346-51; Rolia R et al. Clin Lab 2014; 60 (12): 2089-93).

**Aims:** To provide a recommendation for newborn screening program for SCD in Italy.

**Methods:** A panel of experts was identified by Italian Society of Thalassemia and Hemoglobinopathies (SITE) and Italian Onco-Hematologic Pediatric Association (AIOPEN). The panel has rigorously revised the literature (from 1990 to 2016), the existing recommendations/guidelines of other countries where newborn screening for SCD already exists, and other MADE system (Making All Decisions Easy: Recommendations Assessment, Development and Evaluation) was used to score levels and grades of evidence. The working group produced the draft guideline, and the final version has been revised by external (international) reviewers and the national patients association (UNITED).

**Results:** The recommendations were divided into five sections according to the newborn screening program as well as: 1) testing of newborns and specific screening methods, 2) evaluation of screening results for a definitive diagnosis, 3) enrollment of affected newborns in comprehensive care programs, 4) evaluations of the efficacy of follow-up and interventions, and assessment of the benefit to the patient, family, and society. The on line access for recommendations will be available for clinicians and healthcare providers.

**Summary/Conclusions:** The recommendations for SCD newborn screening program will be an important tool (i) in discussion of strategic newborn screening panel at national level; (ii) to early identify patients to be treated in comprehensive SCD centers and (iii) to produce epidemiological data required for future design of SCD map in Europe.

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Summary/Conclusions: This is the first study highlighting key healthcare practice data for the small but significant number of SCD Day Hospital/Infusion Units around the globe. Our data suggest that among institutions with SCD-DH/UI there is no consensus regarding clinical practice or data collection. We conclude that there is a significant need to further evaluate SCD DH/UI 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 mice, excess free haem has been shown to cause vaso-occlusion that can be reversed by haemopexin, implicating that knowledge on how haemolysis changes haemoglobin production may influence the applicability of clinical use of haemopexin for sickle cell disease and other haemolytic states. Recent studies have reported reduced haemopexin levels in children with sickle cell disease (Santiago et al., 2016) and adults with beta thalassemia (Vinchi et al., 2016) in association with elevated haem levels, thus suggesting regulation of clinical use of haemopexin for sickle cell disease (Santiago et al., 2016) and adults with beta thalassemia (Vinchi et al., 2016) in association with elevated haem levels, thus suggesting that excessive free haem and haemolysis affects haemopexin levels. In sickle cell mice, excess free haem has been shown to cause vaso-occlusion that can be reversed by haemopexin, implicating that knowledge on how haemolysis changes haemoglobin production may influence the applicability of clinical use of haemopexin for sickle cell disease and other haemolytic states. Recent studies have reported reduced haemopexin levels in children with sickle cell disease (Santiago et al., 2016) and adults with beta thalassemia (Vinchi et al., 2016) in association with elevated haem levels, thus suggesting regulation of clinical use of haemopexin for sickle cell disease (Santiago et al., 2016) and adults with beta thalassemia (Vinchi et al., 2016) in association with elevated haem levels, thus suggesting that excessive free haem and haemolysis affects haemopexin levels.

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 haemoglobinopathies (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), but haemopexin was surprisingly elevated in HBSC patients when compared to HbAA (15.08±0.488 vs 8.407±0.864 ng/mL, P= 0.0001), contrary to what has been reported in other haemolytic diseases. Haemoglobin (Hb) was higher in the HbAA group when compared to the HBSC group (12±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 (9.836±948 vs 7.739±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.

Table 1.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>HBSC</th>
<th>HbAA</th>
<th>P-value</th>
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<tr>
<td>Anaemia</td>
<td>20 (50%)</td>
<td>0 (0%)</td>
<td>&lt;0.0001</td>
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<tr>
<td>LDH</td>
<td>15±0.2</td>
<td>8±0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>8,407±684</td>
<td>3,067±334</td>
<td>&lt;0.0001</td>
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</table>

This is the first study highlighting key healthcare practice data for the small but significant number of SCD Day Hospital/Infusion Units around the globe. Our data suggest that among institutions with SCD-DH/UI there is no consensus regarding clinical practice or data collection. We conclude that there is a significant need to further evaluate SCD DH/UI patient-based value, and to develop operational standards / benchmarks to ensure dissemination, adaptability, and sustainability of these alternative care models.
Stem cell transplantation - Clinical

E1496
HIGH PROGNOSTIC VALUE OF PRE-SCT MOLECULAR MINIMAL RESIDUAL DISEASE ASSESSMENT BY WT1 GENE EXPRESSION IN AML TRANSPLANTED IN CYTOLIGIC 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 8670±187 copies/10^4 Abelson-positive bone marrow cells) and before allo-SCT (81±2586 copies/10^6 MRD-WT1-negative and 41±122-44% MRD-WT1 positive cases at the pre-SCT workup).

We evaluated post-SCT Overall Survival (OS), Disease Free Survival (DFS) and Relapse Rate, according to MRD-WT1 pre-SCT status.

Results: Both pre-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 3.19, 95% confidence interval [95% CI]2.0-5.38; 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=0.00073). At univariate analysis, MRD-WT1 negativity before allo-SCT and grade <2 acute GVHD were significant prognostic factors for improved OS and DFS. However, at multivariate analysis, MRD-WT1 negativity before allo-SCT was the only independent prognostic factor for improved OS and DFS.

Summary/Conclusions: These data show that pre-allo-SCT molecular MRD evaluation through WT1 expression is a powerful predictor of post-SCT outcomes (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 secondary prophylaxis (e.g. T-cell infusion, tapering of immunosuppression, azacitidine or new target drugs).

E1497
GOOD IMMUNOLOGICAL RECONSTITUTION IN ADULTS WITH ACUTE LEUKAEMIA AFTER ALFA-BETA TCR/CD19+ DEPLETED HAPLOIDENTICAL STEM CELL TRANSPLANTATION (HSCT)
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Background: Haplo-HSCT based on the infusion of high numbers of T cell depleted (TCD) hematopoietic progenitor cells and no post-transplant immunosuppression controls both graft rejection and GVHD in patients with acute leukemia. One major remaining issue is the delay in the post-transplant immunological reconstitution because of the minimal residual T lymphocytes in the graft and in vivo ATG-linked T cell depletion. Current studies are focusing on rebuilding posttransplant immunity to improve clinical outcomes separating GVHD from favourable donor immune responses. Selective elimination of αβ T cells retains in the graft NK, dendritic cells, monocytes and γδT lymphocytes. Under this approach, a rapid immunological reconstitution and very promising outcome have been reported in pediatric patients.

Aims: With the aims of confirming these results in adults, we tested this approach in adults with acute leukemia.

Methods: Thirty-two patients, median age 51 years (range 19-74), with AML (n=18), ALL (n=5) entered to study. Twenty were in CR (12 CR1; 8 CR2), 12 in advanced-stage disease at transplant. Conditioning consisted of ATG 1.5mg/kg from day -13 to -10, Treosulfan 12 gr/sqm from -9 to -7, Fludarabine 30mg/sqm from -6 to -2 and Thiopeta 5mg/kg on days -4 and -5. PBPCs from haplo-donor (3 mothers, 9 siblings, 13 sons/daughters and 7 cousins) underwer T-cell depletion by CliniMACS. No post-transplant immunosuppression was given. Ganciclovir was given over the conditioning regimen in the 22 patients who were CMV seropositive; L-Amb was used as anti-mold active prophylaxis over the neutrophne phase.

Results: Grafts contained a median of 11x10^6/kg (range 5-19) CD34+ cells, 4.3x10^9/kg CD3+ Tcells/kg (range 1.6-36), 4.9x10^9/kg (range 0.4-62) αβ+ T cells, 4x10^9/kg CD19 +Tcells/kg (range 1.34), 5x10^9B cells/kg (range 1.5-32) and 22x10^9CD56+NK cells/kg (range 5-91). All patient achieved a full donor sustained engraftment. Median time to reach 500 neutrophils and 20,000 platelets was 13 (range 10-18) and 11 days (range 6-30), respectively. Two patients developed and died from severe acute GVHD. One of them had received the highest dose of αβ+ T cells (3.7x10^9/kg) and the second one affected by 6GPDH deficiency experienced a late onset hepatic GVHD. Eight patients had skin limited grade II aGVHD that required short course steroids. Only two patients have so far developed mild cGVHD that recovered completely after steroid and cyclosporin treatment. Tending to confirm our working hypothesis, there was a rapid, sustained increase in peripheral blood T-cell subpopulations (Fig. 1). Naive and memory T-cell subsets increased significantly over the first year after transplantation. B-cell reconstitution was rapid and sustained and immunoglobulin serum levels normalized within 3 months. CMV reactivation and CMV reactivations). One with unfavorable serology (donor negative into recipient positive) developed and died of CMV disease 8 months after transplant. Relapse was the main cause of failure (8/12 in relapse, 3/20 in CR). NRM was 15% (4/12 in relapse, 4/20 in CR), 13 patients survive at a median follow-up of 29 months (range 9-53).

Figure 1.

Summary/Conclusions: The infusion of αβ/CD19-depleted grafts confirmed a fast immunological reconstitution also in adults. Relapse is still a major concern in patients already in relapse at transplantation.

E1498
UNMANIPULATED HAPLOIDENTICAL TRANSPLANTATION CONDITIONING WITH BUSULFAN, CYCLOPHOSPHAMIDE AND ANTI-THYMOMOLOBULIN FOR ADULT SEVERE APLASTIC ANEMIA: GOOD OUTCOME AND PROGNOSIS ANALYSIS
Z. Xu1, L. Xu1, F. Wang1, X. Mo1, T. Han1, W. Han1, Y. Chen1, Y. Zhang1, J. Wang1, Y. Wang1, C. Yan1, Y. Sun1, F. Tang1, X. Zhang1, X. Huang1
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Background: Severe aplastic anemia (SAA) is a life-threatening disorder for which allogeneic hematopoietic stem cell transplantation (HSCT) is the available curative approach. Recently, more and more studies have focused on the feasibility of haplo-identical transplantation in SAA patients because of donor availability.

Aims: To evaluate the outcomes and prognosis of haploidentical hematopoietic stem cell transplantation (HSCT) in adult patients with acquired severe aplastic anemia (SAA), we conducted a retrospective analysis.

Methods: A total of 49 SAA adults received haplo-identical transplantation without in vitro T-cell depletion between May 2011 and December 2016. Forty-six of all cases surviving for more than 28 days achieved donor myeloid engraftment. The median time for myeloid engraftment was 13 (range, 10-21) days and for platelet was 17.5 (range, 7-101) days with the cumulative incidence of 93.88±0.17%. The cumulative incidence of grade II-IV and III-IV acute graft-versus-host disease (aGVHD) were 20.89±0.35% and 4.17±0.08%, respectiv
23 patients showed prompt recovery of neutrophils and platelets. So far, despite engrafted with prompt recovery of granulocytes >0.5x 10^9L at a median of 19 days (0-27) and platelet recovery >50 x 10^9L at day 13 (0-23). Acute graft versus host disease (cGVHD) was observed in 1 donor; in 2 donors grade 3 clotting occurred occurred during (17%), headache or tingling (17%), fatigue/myalgia (17%). CTC grade 3 fatigue was observed in 9 patients.

Results:

Cell subsets including regulatory T cells (Treg), Th1, Th2, and Th17 cells. Median of CD3+ cells in the graft was 22.4 (11-57) x10^9

Discussion: Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

Background: With the goal of immune system reset, autologous hematopoietic stem cell transplantations have been done in patients with multiple sclerosis (MS). Aims: With the goal of immune system reset, autologous hematopoietic stem cell transplantations have been done in patients with multiple sclerosis (MS).

Methods: 131 consecutive patients with MS were autografted in a single center using non-frozen peripheral blood stem cells, on an outpatient basis and conditioned with cyclophosphamide (Cy) and rituximab. The protocol was registered in ClinicalTrials.gov identifier NCT02674217. The PBSCT mobilization schedule was done with Cy and Filgrastim (G-CSF). Intraferon Cy (50mg/kg) was delivered on days -11 and -10. Sucnaguteous G-CSF (10ug/Kg/BID) was delivered on days -9 to -1. The apheresis procedure was performed on day -2. The apheresis objective was to reach at least 1 x 10^6 viable CD34+ cells/kg. As outpatients and after collecting the PBSCT, intraferon Cy (50mg/kg) was delivered over a 120 minute period, on days -2 and -1 followed by MESNA (1000mg/m^2 over a 180 minute period). After the intraferon Cy, oral ondasetron, oral cotrimoxazole, oral fluconazole and oral acyclovir were used in all patients until granulocytes were greater than 0.5x10^9/ L. After the recovery of the granulocytes, patients were given rituximab (375mg/m^2 over a 3h period) and subsequently rituximab (100mg) every two months over a 12-month period. The cumulative dose of Cy is 200mg/Kg.

Results: 80 females and 51 males were included; median age was 47 years. 29 have PPMs, 42 RRRMs, and 61 SPMS. All procedures were started on an outpatient basis and two persons were admitted to the hospital during the procedure. In order to obtain at least 1x10^6/kg viable CD34+ cells, one to four apheresis were performed (median 1). Total number of viable CD34+ cells infused ranged between 1 and 9.6x10^6/kg (median 2.2). Patients recovered neutrophils >0.5x10^9/L absolute granulocytes on median day 9 (range 6 to 12). Two individuals needed red blood cells but none needed platelet transfusions. There were no transplant related deaths and the 125 month overall survival of the patients is 100%. In a subset of 78 persons followed for 3 months or more the EDSS (Expanded Disability Status Scale) was assessed three months after the graft and means diminished from 5.2 to 4.9. The EDSS score improved in 33 patients (42.3%), remained stable in 29 (37.1%) and worsened in 16 (20.5%). Best results of EDSS were found in Relapsing Remitting (82%) and Primary Progressive (80%) type of MS compared to Secondary Progressive (71.4%).

Figure 1.

Summary/Conclusions: It is possible to conduct autotransplants for patients with MS employing non-frozen peripheral blood stem cells and outpatient conduction. Additional information is needed to assess the efficacy of these procedures in the treatment of patients with MS.

E1501

VEDOLIZUMAB IN STERILE REFRACTORY INTESTINAL GRAFT-VERSUS-HOST DISEASE

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1Hematology, Erasmus Cancer Institute Rotterdam, Rotterdam, 2Hematology, VU University Hospital, Amsterdam, 3Hematology, UMCU, Utrecht, Netherlands, 4Hematology, University of Leipzig, Leipzig, Germany

Background: Steroid refractory intestinal graft-versus-host-disease (GvHD) is a serious complication after allogeneic stem cell transplantation (allo-SCT), 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.

E1500

A FEASIBILITY STUDY OF THE FULL OUTPATIENT CONDUCT OF HEMATOPOIETIC TRANSPLANTATIONS IN PERSONS WITH MULTIPLE SCLE- ROSE EMPLOYING AUTOLLOGOUS NON-CRYOPRESERVED PERIPHERAL BLOOD STEM CELLS

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1Hematology, Centro de Hematologia y Medicina Interna, Puebla, Mexico

Background: As outpatients we performed allogeneic T-cells to the intestinal mucosa by inhibiting the binding of T-cell integrin α4β7 to mucosal addressin MadCAM-1.
Patients were endoscopically evaluated at time of GvHD diagnosis and follow-up. Treatment characteristics are provided in Table 1.

**Results:** All 35 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>
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<tr>
<th>Table 1.</th>
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<tr>
<td><strong>Age, median (range)</strong></td>
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<tr>
<td><strong>Duration of symptoms of GVHD, median (range)</strong></td>
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<tr>
<td><strong>Intestinal GVHD grade prior to transplant, median (range)</strong></td>
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<tr>
<td><strong>Histological GvHD grade prior to transplant, median (range)</strong></td>
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<tr>
<td><strong>Doses of prednisone, median (range)</strong></td>
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<td><strong>Observation time, median (range)</strong></td>
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**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 believed to be inhibition of allo-reactive T-cells interacting with intestinal endothelial cells. It is unclear why regulatory T-cells were initially increased in our steroid refractory GvHD patients and subsequently normalized. This might initiate a response to the alloreactive inflammation and subsequent redistribution to affected tissues and/or its resolution after successful treatment.

**E1502**

**RISK FACTORS, OUTCOMES AND CHARACTERIZATION OF ‘AUTOLOGOUS GRAFT VERSUS HOST DISEASE’: THE MAYO CLINIC EXPERIENCE**

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**Background:** Graft versus Host Disease (GVHD) is a common complication of 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 autologous 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 autologous 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 number of prior therapeutic regimens was 2 (range 1-7). GVHD manifestations included gut involvement in 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. In 2 patients who 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 (≥1mg/kg) 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.

**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 DEMYEILINATION AFTER HAPLO-HSCT AND ITS ASSOCIATION WITH THE IGG INTRATECHLAL SYNTHESIS INDEX AND ANTI-MYELIN OLIGODENDROCYTE GLYCOPROTEIN ANTIBODY IN CEREBROSPINAL FLUID**

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**Background:** Haploidentical haemopoietic stem cell transplant (haplo-HSCT) is an up-front and effective therapy for haematological patients, but it usually has many complications such as neurological complications. As one of the neurological complications following haplo-HSCT, immune-mediated demyelinating diseases of the central nervous system (CNS) seriously affect the patient quality of life. However, the incidence, risk factors and pathogenesis of CNS demyelination are not very well understood.

**Aims:** To analyse the incidence, risk factors, and prognosis of CNS demyelination after haplo-HSCT.

**Methods:** A study was conducted in 1,526 patients who underwent haplo-HSCT between January 2013 and June 2016. The definition of CNS demyelination during haplo-HSCT was confirmed by neurologic signs, MRI abnormality corresponding to the neurologic signs, abnormal CSF studies and the presence of systemic GVHD or the response to immunosuppressive therapy (Grauer O et al. Brain. 2010; 133(10): 2852-2865, Chronic graft versus host disease.
Page 243-51, 2009, Thomas’ Hematopoietic Cell Transplantation. Page 766-75, Fifth Edition, 2016, Polman C H et 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 myelin basic protein, CSF and blood anti-myelin basic protein antibody, CSF and blood anti-oligodendrocyte glycoprotein antibody.

Results: Thirty patients developed CNS demyelination after haplo-HSCT. The cumulative incidences of the diseases at 100 days, 1 year and 2 years post transplantation 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 improvement 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 leukocytes, 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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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, adjusted for age, 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 markers.

Results: Patients had a median age of 55 years and HCT-CI scores of 0 (24%), 1-2 (39%), and ≥3 (37%). A cut-off of CrCl<60 ml/min, albumin<3.5 g/dl, and Alk-p>180 IU/l corresponded with 8.8%, 8.3%, and 6.5% of the population, respectively. CrCl and albumin were associated with increased risk and higher cumulative incidence of day-100, 1-year, and 2-year TRM, regardless of whether they were continuous or categorized (Figure-Panel a). A similar pattern was observed with Alk-p, except for day-100 TRM. In a multivariate analysis, a CrCl<60 ml/min and albumin<3.5 g/dl were consistently among the top risk factors for early and late term TRM. Hazard ratios for 2-year TRM of CrCl<60 ml/min and albumin<3.5 g/dl were 2.00 (1.37-2.95) and 2.329 (1.58-3.43), respectively. 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 leukocytes, 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.

E1505
CYTGENETIC AND MOLECULAR RISK FACTORS AT DIAGNOSIS ARE OVERCOME BY WT1 AND FLOW CYTOMETRY-BASED PRE TRANSPLANT MINIMAL RESIDUAL DISEASE ASSESSMENT IN ADVANCED ACUTE MYELOID LEUKEMIA PATIENTS
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Background: Allogeneic bone marrow transplantation (BMT) offers the only chance of cure for patients with advanced acute myeloid leukemia (AML). High levels of pre BMT minimal residual disease (MRD) have been reported to predict relapse risk in patient transplanted in first complete remission (CR), WT1 expression levels and multicolor flow cytometry (MFC) are the most common tools to evaluate MRD.

Aims: Here, we analyzed the role of pre-BMT MRD assessment as predictor for the post-transplant relapse risk in patient transplanted beyond first CR.

Methods: We retrospectively analyzed the outcome of 92 consecutive AML patients receiving allo-BMT in 2nd (CR2) or 3rd CR (CR3). Pre-BMT MRD was evaluated by WT1 expression and MFC. Median age at transplant was 45 years. Disease phase was CR2 in 63 patients (68%) and CR3 in 29 (32%). Risk group according to European Leukemia Net (ELN) at diagnosis was low in 28 patients (30%), intermediate in 44 (48%) and high in 20 (22%). Sixty-six patients (71%) received myeloablative conditioning, whereas 26 (29%) were conditioned with reduced intensity regimen. Stem cell source was HLA-identical sibling in 18 (20%), haploidentical (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 1x10-9 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.

number/Ab1 copy number 250x10^4 was used as cut-off value for abnormal WT1 expression.

Results: Relapse occurred in 30 patients (33%) and two years non-relapse mortality was 29%. Three-year estimate of OS was 47.9% (median19 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 (p<0.03 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 diagnosis (2-years OS of 62.2% and 52.7% among MFC MRD negative patient with ELN risk low or intermediate/high, respectively, Fig.1). The predictive value of MRD resulted independent from all other analyzed variables, although patients with positive MRD undergoing HAPLO BMT had a slightly better outcome. Multivariate OS analysis revealed that MRD status (evaluated by any method) was the only independent predictor of OS (p<0.05 for both). Pre BMT MRD was also a strong predictor of cumulative incidence (CI) of relapse in competitive risk analysis (p<0.01 and p<0.03, respectively, for WT1 and MFC MRD). Multivariate CI of relapse analysis showed that donor source and MRD significantly influenced relapse rate (p<0.05 and p<0.01, respectively).

Aims: There are limited data concerning the impact of specific HLA mismatches on patient outcome and we therefore interrogated the BMT database in order to characterize the impact of mismatch on transplant outcome.

Methods: 937 patients with AML in CR1 or CR 2 underwent transplantation utilizing a RIC regimen using a 9/10 mismatched unrelated donor between 2001-2015. Of these 264 were transplanted using a donor mismatched at HLA-A, 127 were mismatched at HLA-B, 292 mismatched at HLA-C, 180 mismatched at HLA-DQ and 74 mismatched at HLA-DRB1. 85% of patients received in vivo T cell depletion.

Results: The 2 year leukemia free survival (LFS) for the whole cohort was 45% and the 2 year overall survival (OS) was 50%. The corresponding non-relapse mortality was 26% and relapse incidence 28%. 45% of 22 patients developed Grade 2-4 acute GVHD and 14% chronic extensive GVHD. In Cox analysis age, adverse karyotype and patient CMV seropositivity were correlated with decreased LFS and OS. There was no significant difference in LFS or OS between patients transplanted from donors mismatched at HLA-A, B, C, DRB1 and DRQ respectively. Of note HLA-C mismatch was associated with a lower incidence of acute GVHD but this did not translate into a survival difference. Patient:donor CMV mismatch was associated with a marked increase in transplant related mortality and concomitant reduction in LFS and OS in all groups studied.

Summary/Conclusions: To our knowledge this is the largest to date studying the impact of specific HLA mismatch on the outcome of adults undergoing a RIC allograft from an adult unrelated donor. Recipients of HLA-A, B, C, DRB1 and DRQ mismatched allografts demonstrated equivalent outcomes. Patient:donor CMV disparity is an important adverse prognostic factor in HLA mismatched transplants. These data have the potential to inform donor selection in allo-mandatory adults with AML undergoing a RIC allograft who lack a 10/10 matched donor.

E1506 IMPACT OF ALLELE SPECIFIC PATIENT:DONOR HLA DISPARITY ON OUTCOME OF REDUCED INTENSITY TRANSPLANTS PERFORMED USING HLA MISMATCHED UNRELATED DONORS: ON BEHALF OF THE ALWP OF THE EBMT

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Background: Relapse remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%. Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL.

Aims: To determine the efficacy and safety of IFN-α-2b pre-emptive therapy for acute leukemia(AL) patients with relapsing tendencies after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Methods: Retrospectively analyzed 986 acute leukemia patients undergoing allo-HSCT from Jan, 2006 to Mar, 2014 in our hospital. After allo-HSCT, 986 AL patients were periodically monitored the minimal residual disease(MRD) including: bone marrow smear, leukemia-associated immunophenotype (LAIP), leukemia specific or related fusion genes, and donor chimerism through multi-parameter detection to evaluate disease status. Patients were given IFN-α-2b 2-3 million units / day by subcutaneous injection for preemptive treatment once a relapse tendency was detected, such as: increasing proportion of blasts in bone marrow between 3–5%, or MRD>1.0x10^3, or leukemia specific fusion gene transfrom negative to positive, or dynamic incressing copy number of WT1 more than 200 copies/10^4 abl, or decreasing of donor chimerism(≤ 90%). Gene expression.

Results: There were 98 patients who presented increasing tendency of MRD once a relapse tendency was detected, such as: increasing proportion of blasts in bone marrow between 3–5%, or MRD>1.0x10^3, or leukemia specific fusion gene transfrom negative to positive, or dynamic incressing copy number of WT1 more than 200 copies/10^4 abl, or decreasing of donor chimerism(≤ 90%). Gene expression.

There are limited data concerning the impact of specific HLA mismatches between two groups. For the 31 patients who received IFN-α-2b pre-emptive therapy(IFN group), the median time of IFN-atreatment was 60 days (range: 5–720 days), Twenty five patients had responded to the treatment without progressing to hematological relapse (response rate 80.6%). 2 patients developed to hematological relapse again after temporary response; 3 patients had no response and eventually progressed to hematological relapse. Regarding 67 patients who received non-IFN-α-2b therapy including: withdraw immunosuppressant, traditional DLI or DC-CIK immunotherapy, and 67 patients received non-IFN-α-2b therapy such as: withdraw immunosuppressant, traditional DLI or DC-CIK immunotherapy.

Results: There were no significant differences in disease characteristics between two groups. For the 31 patients who received IFN-α-2b pre-emptive therapy(IFN group), the median time of IFN-atreatment was 60 days (range: 5–720 days), Twenty five patients had responded to the treatment without progressing to hematological relapse (response rate 80.6%). 2 patients developed to hematological relapse again after temporary response; 3 patients had no response and eventually progressed to hematological relapse. Regarding 67 patients who received non-IFN-α-2b therapy including: withdraw immunosuppressant, traditional DLI or DC-CIK immunotherapy, and 67 patients received non-IFN-α-2b therapy such as: withdraw immunosuppressant, traditional DLI or DC-CIK immunotherapy.

Discussion: Relapse still remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%. Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL.

E1507 PRE-EMPTIVE THERAPY WITH IFN-α-2B FOR ACUTE LEUKAEMIA PATIENTS WITH HIGH RISK OF RELAPSING TENDENCY POST ALLO-HSCT

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Background: Relapse remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%. Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL.

Aims: To determine the efficacy and safety of IFN-α-2b pre-emptive therapy for acute leukemia(AL) patients with relapsing tendencies after allogeneic hematopoietic stem cell transplantation (allo-HSCT).

Methods: Retrospectively analyzed 986 acute leukemia patients undergoing allo-HSCT from Jan, 2006 to Mar, 2014 in our hospital. After allo-HSCT, 986 AL patients were periodically monitored the minimal residual disease(MRD) including: bone marrow smear, leukemia-associated immunophenotype (LAIP), leukemia specific or related fusion genes, and donor chimerism through multi-parameter detection to evaluate disease status. Patients were given IFN-α-2b pre-emptive therapy once a relapse tendency was detected, such as: increasing proportion of blasts in bone marrow between 3–5%, or MRD>1.0x10^3, or leukemia specific fusion gene transfrom negative to positive, or dynamic incressing copy number of WT1 more than 200 copies/10^4 abl, or decreasing of donor chimerism(≤ 90%).

Discussion: Relapse still remains the most frequent cause of treatment failure and mortality. According to the data of CIBMTR, relapse has become the leading cause of death following allo-HSCT. The high risk patients with more advanced disease, have a relapse rate of 40–80%. Therefore, prevention and treatment of relapse post allo-HSCT is the most likely approach to improve survival of these patients. It is well known that IFN-α had been widely used in the field of antitumor. Recently it is shown that IFN-α also play an important role in immune modulation to enhance the effect of GVL.
free survival rate (LFS) of IFN group were 47.0%±13.9% and 38.7%±13.1% respectively. However, the 5-yr OS and LFS of non IFN group were 14.5%±10.7% and 12.5%±9.4% respectively. The difference were significantly (P<0.000, P<0.002 respectively). Patients with GVHD had significantly better response than patients without GVHD (88.9% vs 53.8%, P=0.043, P < 0.05).

Summary/Conclusions: IFN-α-2b pre-emptive therapy can effectively prevent high-risk patients with relapsing tendencies for disease progression post allo-HSCT. Further large-scale investigation is warranted.

E1508
PREDICTING SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION. THE GATMO SCORE
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Background: Several attempts to predict mortality after autologous stem cell transplantation (ASCT) have been made, like Hematopoietic Stem Cell Transplant Comorbidity Index (HCT-CI) score, originally described by Sorror for allogeneic HSCT. There is no score applicable to the clinical practice that integrates comorbidities with other patient characteristics.

Aims: To describe a comprehensive score that combines comorbidities with other factors and analyse the impact of this score in OS and NRM after ASCT in a cohort of patients transplanted in Argentina.

Methods: We retrospectively reviewed a cohort of 1453 medical records of adult patients who received an ASCT in our centres between October 2002 and August 2016, for Multiple Myeloma or Lymphoma. We compared NRM and Relapse with CI, OS with KM and long term MVA with fine-Gray or Cox regression. We included in the score all the factors that remained significant after MVA for NRM, and assigned a score of 1 if the Hazard ratio (HR) was around 2 (1.5-2.5) and 2 if it was around 3 (2.6-3.5).

Results: Mean age was 50.7 years (range 15-74); 57% were male, 52% had Multiple Myeloma, 29% Non Hodgkin Lymphoma and 19% Hodgkin Lymphoma. Forty-seven percent were in CR, 50% in PR and 3% SD/PD; 14% received three or more chemotherapy lines before transplant (heavily pre-treated). Regarding comorbidities, 62% had low HCT-CI score (score 0), 26% intermediate-risk (1-2) and 12% high risk (≥3). Median follow up was 1.1 years (range 0.1-8.5). Early NRM (day 100) was 2.8%, long term NRM (1-3 years) was 1.1-1.1% and OS (1-5 years) was 93-50% respectively, p<0.001. A Hazard ratio for NRM increased proportionally with the score (1 2.2, 2 3.5, 3 4.3 and ≥5 6.3). Patients were grouped as low risk (LR) with a score 0, 1 (1.5-2.5) and 2 if it was around 3 (2.6-3.5).

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 value <0.05 was considered significant.

Results: 169 patients received HDC-ASCT. The median age was 58 years (23-74). Patient characteristics are shown in the table (See Image). Thirteen patients (7.6%) required ICU admission at a median of 14 days post cell infusion (range 5-85), with all patients having a neutrophil count <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, 1; haemofiltration; 2) and 2 required only management of
fluid balance. Six patients required multi-organ support (non invasive ventilation/ intubation, haemofiltration and inotropic support) and all died. Four patients died within 30 days of HDC-ASCT and had not engrafted neutrophils at the time of death. Two patients died late at day +120 and day +93 post HDC-ASCT. The latter had both successfully engrafted neutrophils but subsequently became neutropenic. Causes of death were neutropenic sepsis (3), cerebrovascular accident (1) and acute respiratory distress syndrome (ARDS) versus host disease (1). By univariate analysis none of the baseline parameters, comorbidities or conditioning regimens were predictive of ICU admission. The only parameter for which there was a trend for significance was baseline cardiac ejection fraction (EF) <50% (p=0.05). Three patients that required ICU had an EF <50% and 2 were on heart failure medications prior to HDC-ASCT. Two of these 3 patients died.

Summary/Conclusions: In this retrospective series, the risk for ICU admission and death following HDC-ASCT was 7.6% and 3.5% respectively. All patients requiring more than one organ support died. The only predictor of ICU admission was baseline cardiac ejection fraction but this would need confirmation in a larger series. Patient selection remains challenging with no definite way to predict ICU admission or death.

E1510

AUTOLOGOUS STEM CELL TRANSPLANTATION WITH BENDA-EAM (BENDAMUSTINE, ETOPOSIDE, CYTARABINE, MELPHALAN) IN AGGRESSIVE NON HODGKIN AND HODGKIN’S LYMPHOMA

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Background: Autologous Stem Cell Transplantation (ASCT) is standard of care in relapsed diffuse large B-cell lymphoma (DLBCL) and other lymphoproliferative disorders (relapsed Hodgkin’s disease, 1st line mantle cell lymphoma (MCL) or T-cell lymphoma). BCNU, Etoposide, Ara-C, Melphalan (BEAM) is a standard conditioning regimen, but BCNU is known to be associated with interstitial pneumonia (range 2 to 20%) and an increased risk of death compared with other regimens.

Aims: Therefore a less toxic conditioning protocol might improve the results in lymphoma patients. Bendamustine showed promising results in B- and T-cell lymphoma and dose escalation is safe and feasible. Here we report promising results with bendamustine replacing BCNU in the BEAM regimen described as Benda-EAM, previously published in a phase two dose finding study (Visani, Blood 2011).

Methods: Forty-one patients with Hodgkin’s (HL) (n=9) or Non-Hodgkin (n=32) lymphoma were consecutively treated with Benda-EAM (bendamustine on two consecutive days at a dose of 200mg/m² per day). Eleven patients were diagnosed with DLBCL, ten patients with MCL, six patients with follicular lymphoma (FL), three patients with T-cell lymphoma (TCL) and two patients with greyzone lymphoma (GZL). Twenty-seven patients were male and fourteen female with a median age of 52 years (range 22-71) and 25% were above the age of sixty.

The median lines of previous therapies were 2 (range 1-4).

Results: All patients had chemosensitive disease and before transplantation, 34 patients (83%) were in complete (CR) and 7 (17%) in partial remission (PR). A median number of 4,20*10⁶ CD34+ cells/kg (range: 1,60-13,30) were infused. Three patients showed progression after a median time of 7 months after transplantation. Until today nine patients received an additional allogeneic transplantation. 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, aGVHD and concomitant aGVHD after allo-HSCT remains not well understood.

Aims: To explore the possible risk factors for the occurrence and mortality of TMA with concomitant aGVHD and to investigate outcomes and treatments of this disorder after allo-HSCT.

Methods: This study was based on patients who underwent allo-HSCT at Peking University People’s Hospital from January 2008 to December 2016. We included patients who showed refractory diarrhea and underwent enteroscopy and biopsy. The diagnosis of TA-TMA and aGVHD were mainly based on the probable-TMA criteria (Byung-Sik Cho et al. Transplantation 2010;90:918-926) and endoscopic appearance and histologic findings (Thomas Hematopoietic Cell Transplantation, Fifth Edition, 2016), respectively. The potential factors affecting TMA with concomitant aGVHD occurrence and markers associated with the death of these patients were identified using univariate and multivariate Cox analysis. The cumulative incidence of relapse, non-relapse mortality (NRM), overall survival (OS) and disease-free survival (DFS) were estimated using the Kaplan-Meier method and were compared by the log-rank test.

Results: Among all 3,992 allo-HSCT recipients, 276 patients showed refractory diarrhea and underwent enteroscopy; of these patients, 50 (1.3%) were diagnosed with TMA with concomitant aGVHD and were enrolled in the case group, and 150 (5.8%) 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 1% (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, its clinical significance has, to date, been to assess 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

Figure 1.
CBFB-MYH11 transcript levels at HSCT on transplant outcomes in AML patients with CBF abnormalities.

**Methods:** We included 61 AML patients with CBF at diagnosis that underwent their first HSCT in complete remission (CR) from January 2007 through May 2016. Of 61, 19 (31%) had t(8:21) chromosomal translocation and 42 (69%) inv(16)(p13; q22). Disease status at HSCT was CR1 in 19 (31%) and CR2 in 42 (69%), respectively. Furthermore, bone marrow and peripheral blood bone marrow (62%), 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%), mismatched 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)<100.

**Results:** MRD at 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 ALLOGENIC STEM CELL TRANSPLANTATION IN ADULT SEVERE APLASTIC ANEMIA WITH ABNORMAL CYTOGENETICS AT DIAGNOSIS**

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**Background:** Cytogenetic abnormalities (CAs) have been reported at the time of diagnosis of acquired aplastic anemia (AA), up to approximately 4-15%. Considering evolution into clonal hematologic disorders and difficulty between AA and hypocellular MDS, clinical implications of CAs in AA is important.

**Aims:** In this study, we investigated long-term outcome of allogeneic stem cell transplantation (SCT) in adult severe AA (SAA) patients with abnormal CAs at diagnosis.

**Methods:** Total of 19 patients with abnormal CAs at diagnosis who underwent allogeneic SCT at our institution between 2003 and 2015. Morphologically hypocellular bone marrow with dysplastic cells was considered as hypocellular 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 +Y, -7, +9. Two patients had two or more CAs. Seven male and 12 female patients with a median age of 41 years (range, 20-59 years) were included. Patients had received SCT from HLA-matched sibling (n=12), unrelated (n=5), or haplo-identical donor (n=2). After a median follow-up of 66.3 months (range, 69-156.3), the 5-year estimated OS rates were 94.7±5.1%. One patient died on reverse-transcribed RNA for the

**E1514**

**PROGNOSTIC VALUE OF PET/CT PRIOR TO AUTOLOGOUS HCT IN RELAPSED / REFRACTORY LYMPHOMA**

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**Background:** Positron Emission Tomography/Computed Tomography (PET/CT) is emerging as a powerful prognostic tool in the management of Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL). A number of retrospective single center cohorts have reported that a positive PET/CT prior to autologous Hematopoietic Stem Cell Transplantation (HCT) is an adverse factor associated with higher relapse risk. However, important heterogeneity is noted in these studies due to differences in timing of PET/CT prior to HCT as well as different metabolic activity threshold (i.e. Deauville ≤2 vs ≥3). At our institution, we perform PET/CT within 4 weeks prior to HCT and after all intended salvage therapy is administered.

**Aims:** We sought to further investigate the prognostic value of PET/CT in relapsed / refractory lymphoma patients prior to HCT.

**Methods:** After due IRB approval, patients who received autologous HCT at our institution for relapsed / refractory lymphoma between 2010 - 2016 were identified. All variables were retrospectively extracted. PET/CT reports were reviewed and metabolic activity was assigned per Deauville criteria. Patients with primary CNS lymphoma were excluded. Refractory disease indicates disease progression prior to completing planned first line therapy. Categorical and continuous variables were compared using Chi-squared and Wilcoxon tests, respectively. Time to event analysis was computed using the method of Kaplan and Meier with log ranks. Competing events were computed using Grey’s method considering non relapse mortality as a competing event for relapse. Analysis was computed using JMP software, version 11.
was 109 days (55-395) vs 271 days (55-449) for PET positive vs PET negative patients, respectively. Mortality post relapse was lower than 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, immunotherapy prior and during lymphocytes (allo-DLI) in patients with MRD-positive after allo-HSCT may 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. In total, 3 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 (53%) 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 rereseed intervention were 58.9% versus 54.3% (P=0.862), 69.3% versus 78.1% (P=0.361), and 44.4% versus 35.1% (P=0.489) in the DLI and 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 2-year OS (logistic) in allo-HCT recipients matched for age at the HSCT, underlying diseases, and the year of the HSCT. However, the impact of HCT-CI on clinical outcomes in single unit umbilical cord blood transplantation (UCBT) has not been investigated extensively.

Aims: The purpose of this single-center retrospective study was to investigate the impact of the 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=50) and the non-UCBT group (n=93). 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%, 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=2.8) and higher NRM (p=0.015; hazard ratio=3.1) for the non-UCBT group, but not for the UCBT group. There was no significant difference in the cumulative incidences of grade 2 to 4 acute GVHD between the non-UCBT group (41%) and the UCBT group (33%, P=0.51). Similarly, there was no significant difference in the cumulative incidences of grade 3 to 4 acute GVHD between the non-UCBT group (8.8%) and the UCBT group (6.1%, P=0.80). The cumulative incidence of extensive chronic GVHD was significantly higher in the non-UCBT group compared with the UCBT group. (38% vs 3.8%, P=0.001) Although not significant, patients in the non-UCBT group were more likely to have the systemic steroid therapy compared with those in the UCBT group. (54% vs 34%, P=0.084).

Figure 1.

Summary/Conclusions: UCBT showed good OS with the low incidence of NRM even in patients with high HCT-CI scores. These results indicate that a single unit umbilical cord blood might be a promising stem cell source for patients with multiple comorbidities. Further studies are needed in order to validate these results.

E1517

LOW DOSE POSTTRANSPLANTATION CYCLOPHOSPHAMIDE CAN ENHANCE THE PROTECTIVE EFFECT OF ATG /G-CSF ON GVHD: RESULTS OF A PHASE II PROSPECTIVE TRIAL

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Background: Anti-thymocyte globulin (ATG)/granulocyte colony-stimulating factor (G-CSF)-represented regimen produces essentially universal engraftment with limited relapse and favorable survival, albeit with relatively high rates of graft-versus-host disease (GVHD), especially after HCT from maternal donor or collateral relatives. While use of high-dose, post-transplant cyclophosphamide (PT/Cy) results in low rates of GVHD and favorable immune reconstitution, although with higher rates of relapse and somewhat high rates of graft failure. Thus, novel strategies are needed to refine each approach: under Beijing protocol including ATG and G-CSF, reducing GVHD without abrogating GVL effect is a major priority.

Aims: In order to benefit patients at high risk of developing GVHD without abrogating engraftment and GVL effects, we sought to develop a novel procedure in TCR haplo-HCT with intensified conditioning containing ATG and G-CSF followed by lower-dose of PT/Cy. In addition, the current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by the new strategy.

Methods: We performed a prospective pilot study of HLA haploidentical cell transplantation from maternal or collateral relative donors with intensified conditioning including G-CSF and ATG, followed by two lower doses of PT/Cy (14.5mg/kgx2 doses; designated as Group A). Outcomes were compared with those of 160 controls from matched-pair analysis who undergone haploidentical HCT from other donors than mother or collateral relatives at the same time period (Group B) as well as with those of 46 historically reported HCT from mother or collateral relatives at earlier time period (Group C). In addition, the current study attempt to establish a murine model and focus on Treg cells to clarify the immunological mechanisms for GVHD prevention by
the new strategy. Trial registration: The study is registered at www.clinicaltrial.gov as NCT02412423.

**Results:** We found that low dose PT/Cy combined with ATG could alleviate GVHD in mice and could increase the number of Treg cells while having no effects on CD4+ or CD8+ T cells. A total of 40 patients with myelodysplastic syndrome (MDS) and leukemia undergoing haploidentical HCT from maternal or collateral donors were enrolled in the study. The cumulative, 100-day incidence of acute GVHD, grades II-IV, in Group A (17%; 95% CI, 5%–29%) was significantly lower than both that in Group B (33%; CI, 25%–41%; P=0.04) and that in Group C (56%; CI, 42%–70%; P<0.001). The 1-year probabilities of NRM (5%; CI, 0%–12%), OS (84%; CI, 88%–100%), and LFS (83%; CI, 70%–96%) in Group A were similar to that in Group B, but was significantly lower than that of Group C (28%; CI, 15%–41%; P=0.006; 65%; CI, 51%–79%; P=0.02; and 65%; CI, 51%–79%; P=0.04; respectively).

**Summary/Conclusions:** Low dose PT/Cy can enhance the protective effect of ATG/G-CSF on GVHD. Conditioning with ATG/G-CSF and low-dose PT/Cy might be a feasible option for patients undergoing HLA haploidentical, T-cell replete HCT, in particular for those with high GVHD risk.

**E1518**

**HEPATITIS B REACTIVATION IN HEMATOPOIETIC STEM-CELL TRANSPLANTED PATIENTS: 22 YEARS EXPERIENCE OF A SINGLE CENTRE**

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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:** Patient files and electronic records of 561 patients who received HSCT between 1994 and 2015 at the Bone Marrow Transplantation Centre of Cerrahpasa Medical Faculty were retrospectively evaluated. A total of 66 patients with HBsAg (n=15; 12 autologous, 3 alcoholic) and anti-HBc positivity (n=51; 29 autologous, 22 alcoholic) were included in the study. Cases were grouped according to transplant types (autologous or allogenic) and anti-HBc positivity (HBs positive or negative) to calculate relative risks and cumulative incidences of HBV reactivation.

**Results:** Four (26%) of the 15 patients with HBsAg positivity showed HBV reactivation in an average of 13 months following HSCT. While cumulative incidence of reactivation was 7% at day 60, it went up to 16% and 44% at days 270 and 730 following HSCT, respectively. In Anti HBc IgG positive group, alcoholic HSCT (n=22) was a higher risk factor for reactivation (31.8%) than autologous HSCT (n=29, 6.8%). Relative risk of reactivation in the allo-transplanted patients who were anti-HBC IgG positive and anti-HBs negative was 6.8 when compared to anti-HBC positive patients (n=9, 55%; vs n=13, 10%; 95CI: 1.34-65). Cumulative incidence of reactivation in anti-HBc IgG positive anti-HBs negative patients (isolated anti HBc IgG positivity) was 11% at day 10, 33% at day 133, 50% at day 400 and going up as high as 75% at day 940.

**Summary/Conclusions:** The results of our study indicate that HBsAg positive patients undergoing autologous or alcoholic HSCT should receive prophylaxis at least one year posttransplant. Anti-HBc IgG positive patients carry the risk of reverse seroconversion, with receivers of allogenic 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 allo- genetic 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**

**ALLOGENIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION FROM HAPLOIDENTICAL DONOR WITH POST-TRANSPLANT CYCLOPHOSPHAMIDE WAS RELATED TO LESS INPATIENT COST COMPARED TO CORD BLOOD TRANSPLANTATION**

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**Background:** The number of allogenic 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 allogenic HSCT in order to clarify the impact of donor sources and other clinical factors on these outcomes.

**Methods:** Patients (n=134) with hematological malignancies who underwent allogeneic HSCT between January 2013 and December 2016 in University of Tsukuba Hospital were included. The days of the initial hospitalization (from the beginning of the conditioning regimen to discharge), the whole initial inpatient costs and the costs of transfusion during the initial hospitalization was retrospectively analyzed.

**Results:** The median age of the patients was 46 (range, 16–67) years. The diagnoses were AML (n=66), ALL (n=31), MDS (n=17), lymphoma (n=11), and others (n=9). Twenty-seven patients were transplanted from MRD, 37 from MUD, 22 from haplo with PT/CY, and 48 with single-unit CB. The median initial inpatient cost was €49179 (IQR, 37030–66923), the median transfusion cost was €11500 (IQR, 9500–15250), and the median length of initial hospitalization was 55 (IQR, 44–75) days. CB showed significantly higher inpatient cost (median, €66852) than haplo (median, €49085, P=0.008 vs CB), MRD (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), MUD (median, €12699, P=0.001 vs CB), and MUD (median, €13118, P=0.001 vs CB). The median hospitalization days were 67 in CB, 61 in haplo (P=1.0 vs CB), 46 in MUD (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/inter/high/very high), donor source (MRD, 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 4 is a potent anti-inflammatory agent. It is a transcription factor belonging to the nuclear hormone receptor super family which may be participating in aGVHD.

Aims: To explore the role of PPARy 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 PPARy, IFNy, 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 PPARy agonist.

Results: Among 65 patients after HSCT, aGVHD occurred in 45 patients. Expression of PPARy mRNA in healthy controls were significant higher than that in patients after allo-HSCT within 90 days (P<0.05). The expression of PPARy mRNA held steady in non-GVHD patients within 90 days after allo-HSCT, and was significantly lower in GVHD group than in non-GVHD group (P<0.05). PPARy expression in severe aGVHD (grade II) was 41.7% and 35.5% (P=0.37), while mild aGVHD (grade I to 2) patients (P<0.05). The expression of IFNy and T-bet increased in aGVHD patients and were negatively correlated with PPARy mRNA expression (P<0.05). The expression of MLR shows that PPARy agonist rosiglitazone above concentration of 25μM had dose-dependent inhibition effect to proliferation of lymphocytes.

Summary/Conclusions: Low expression of PPARy is associated with aGVHD occurrence and degree. PPARy agonist can inhibit the proliferation of lymphocytes, which may be a new way to treat aGVHD.

E1521

HAPLOIDENTICAL TRANSPLANTATION WITH MYELOABLATIVE CONDITIONING REGIMEN COULD SERVE AS AN OPTIONAL SALVAGE THERAPY FOR YOUNGER PATIENTS WITH REFRACTORY OR RELAPSED NON-HODGKIN LYMPHOMA

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Background: Allogeneic hematopoietic stem-cell transplantation (allo-HSCT) has a well-established role in the treatment of refractory or relapsed (R/R) aggressive non-Hodgkin lymphoma (NHL). However, whether patients with R/R aggressive NHL, in the absence of appropriate HLA-matched donors, can benefit from haploidentical hematopoietic stem cell transplantation (haplo-HSCT) is yet to be elucidated. Herein, we evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

Aims: To evaluated clinical outcomes of haplo-HSCT in patients with R/R aggressive NHL.

Methods: 23 patients with R/R aggressive NHL who had undergone haplo-HSCT in our center between January 2004 and December 2015 were included, and data were retrospectively analyzed. 25 patients with R/R aggressive NHL who received HLA-matched HSCT during the same period constituted the control group for this analysis. All patients received myeloablative conditioning (MAC) regimen. Antithymocyte globulin (ATG) was administered to prevent graft-versus-host disease (GVHD). The median age of patients who underwent allo-HSCT was 33 years (range, 16-58). Twenty-three patients had received transplant from haploidentical donors, while twenty-five patients received transplant from HLA-matched donors, of which included 13 ISD and 12 MUD. Chemoresistant disease at transplantation was more common in the haplo-HSCT group as compared to that in the HLA-matched HSCT cohort (P=0.005). No significant between-group differences were observed with respect to distribution of age and sex, histological subtype, bone marrow involvement, aaiPI score, chemotherapy regimen and relapse after ASCT.

Results: Median age of patients at allo-HSCT was 33 years (16-58). Over a median follow-up of 23 months, 27 out of the 48 patients (56%) were alive. Progression free survival (PFS) rate at 2-years in the haplo-HSCT and HLA-matched HSCT groups was 52.1% and 56.6%, respectively (P=0.75); 2-year overall survival (OS) rate was 52.8% and 57.8%, respectively (P=0.83). Cumulative incidence of relapse (IR) was 41.7% and 35.5% (P=0.37), while non-relapse-mortality (NRM) was 21.7% and 35.0%, respectively (P=0.32). Collectively, these results showed no significant difference with respect to major allo-HSCT endpoints between the haplo-HSCT and HLA-matched-HSCT groups. On multivariate analyses, older age (>45 years), primary chemorefractory disease and occurrence of grade III-IV aGVHD were associated with poor prognosis in both groups. Likewise, the most important factors that influenced the overall survival rate in the haplo-HSCT group were age and occurrence of grade III-IV aGVHD.

Summary/Conclusions: Haplo-HSCT with MAC regimen could serve as an optional salvage therapy with outcomes comparable to those of HLA-matched HSCT, particularly in younger patients with R/R NHL without appropriate donors.

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) AND INV(3)(q21;q26.2)/T(3;3)(q21;q26.2)

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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)] is categorized as AML with recurrent genetic abnor-
mality 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) remain unclear.

Aims: We retrospectively examined the impact of inv(3)(t;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), who were aged ≥16 years and underwent their first transplantation between January 2000 and December 2014. We analyzed outcomes assessed 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 were analyzed: age, sex, disease status at HSCT, some 7 and type of 3q abnormality.

Results: Of 15025 patients with AML who were aged ≥16 years and who under-
gone first transplantation, inv(3)(t;3) (q21;q26.2) (t;3)(q21;q26.2) was identified in 66 patients. The median age was 46 years (range, 16-72 years). Of the 66 patients, 10 (15.2%) were in first complete remission (CR1) at allo-HSCT, 54 (81.8%) were in non-CR, and the disease status of two patients was unknown. The probabilities of 2-year OS, relapse, and NRM were 27.8% (95% CI 22.4%-32.5%), 64.2% (50.4%-78.0%), and 21.1% (11.8%-32.3%), respectively. Multivariate analysis revealed an age that ≥50 years (HR, 2.05; 95% CI, 1.06-3.99; P=0.03) was significant risk factors for poor OS. Non-CR at transplantation (HR, 2.55; 95% CI, 0.94-6.93; P=0.07), and reduced conditioning intensity...
Summary/Conclusions: These findings revealed that AML with inv(3)(t;3;3) had dismal outcome even after allo-HSCT. Multivariate analysis suggested that a myeloablative conditioning regimen might improve the transplantation outcome.

E1523

PHARMACOKINETICS (PK) OF PROPYLENE GLYCOL-FREE MELPHALAN HCL (PG-FREE MEL) IN MULTIPLE MYELOMA (MM) PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION (AHCT)

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Background: Melphalan (MEL) is the most commonly used conditioning agent in AHCT for MM and exhibits a dose response relationship (Nath CE Br J Clin Pharmacol. 2010 May; 69(5):484). PG-free MEL (EvomelaTM) has longer stability in solution, results in a slightly higher systemic exposure compared with standard MEL and eliminates propylene glycol administration during high dose melphalan-based conditioning. This agent was shown to be bioequivalent to conventional melphalan leading to successful myeloblation and engraftment in MM pts receiving AHCT with no transplant related mortality or unexpected toxicity leading to its FDA approval (Hari P Biol Blood Marrow Transplant. 2015 Dec; 21(12):2100). Published studies thus far have used PG-free MEL in 2 consecutive daily doses of 100mg/m²/day while a single daily conditioning dose of 200mg/m² (MEL200) is most commonly used in clinical practice.

Aims: Determine the safety and PK variability of high dose PG-free MEL 200mg/m² in patients undergoing AHCT for MM

Methods: Open-label phase II study in which 10 serial blood samples at specific time points for the PK evaluation of melphalan were collected immediately prior to and after receiving single 200mg/m² dose of PG-free MEL on day -2 as a 2mg/ml solution. The primary objective was a descriptive analysis of melphalan PK while secondary objectives included the response rates, engraftment and the toxicity and safety profile of PG-free MEL conditioning.

Results: As of Feb 2017, a total of 24 pts. were enrolled (63% male) with a median age of 67 years (range 46-72), including 23 (96%) who received upfront AHCT and 1 (4%) after relapse (Figure 1). High-risk cytogenetics was present in 6 (25%) pts 25% were in ISS stage 3. Disease status at transplant was complete remission (CR) in 26 (66%), very good partial remission (VGPR) in 12 (30%) and PR in 6 (16%). AHCT was performed entirely as outpatient in 25%.

PK data are available for the first 12 pts at this time. Wide variability in MEL exposure was noted with maximum plasma concentration (Cmax) of 10,100 ng/ml, median Cmax 7750ng/ml (range, 5220-10,100) and median area under the concentration- time curve (AUC) of 561500 ng.min/ml (range, 771000-254000). Mean AUC was 549000 (±155000). No grade 4 non-hematologic toxicities or gastrointestinal toxicities were observed including in patients with Cmax >10,000 (upper quartile of distribution) or AUC>625000. All patients are alive and post-transplant responses in those with at least 100 days of follow up indicate sCR/CR in 60% and VGPR in 30%.

Figure 1.

Summary/Conclusions: PG-Free MEL can be safely administered as a single 200mg/m² 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 (thiotepa [40mg/m² x four days], etoposide [200mg/m² x four days], cyclophosphamide [60mg/kg x one day], cytarabine [200mg/m² x four days] and melphalan [80mg/m² x two days]) or BEAM (carmustine [300mg/m² x one day], etoposide [200mg/m² x four days], cytarabine [200mg/m² x four days], 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 innate immune response genes mutation status to the duration of neutropenia after autologous transplantation of peripheral blood stem cells in patients with multiple myeloma.

Methods: The study included 19 patients with multiple myeloma at the age of 32 to 67 years (median - 52 years) who underwent autologous transplantation of hematopoietic stem cells after conditioning regimen with high-dose melphalan. Among surveyed: 8 men and 11 women. In accordance with staging for Durie-Salmon (DSS) system in patients following stages of MM were installed: stage 1A in one patient (5.2%), stage 2A - in 12 patients (63.2%), stage 2B - in two patients (10.5%) and stage 3A - in four patients (21.1%). In the 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 (rs5743866), IL1β (rs2069762), IL4 (rs2245250), IL6 (rs1800795), IL10 (rs1800871), IL17A (rs2275913), CD14 (rs34424920), TMAF (rs1800629), FCGR2A (rs1801274) was performed by polymerase chain reaction with allele-specific primers (Lifei, Russia) at the time of diagnosis.

Results: Depending on the duration of the neutropenia period all examined are divided into two groups. The first group included 10 patients with MM who have early observed recovery (within the first 13 days, 11-13 days), the number of leukocytes ≥1000 cells per ml after auto-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: 0.60-288.34, p=0.03) and with a predominance of heterozygous mutant allele C of the gene IL1β at position -31 (OR 8.17, 95%CI: 1.03-67.94, p=0.04).

Summary/Conclusions: Our findings point to immune response genes involved in the rate of recovery of hematopoiesis in MM patients after autologous HSCT. Identification of the wild-type allele in intron gene IL17A (G-197A) and mutant allele in intron gene IL1β (T-31C) will predict the risk of prolonging the period of agranulocytosis and, consequently, the risk of post-transplant complications, and develop a personalized strategy of managing them.

E1527

SUCCESSFUL TREATMENT WITH GRANULOCYTE TRANSFUSION AND EARLY NEUTROPHIL ENGRAFTMENT IN ALLOGENIC TRANSPLANT PATIENTS WITH FEBRILE NEUTROPHENIA

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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 (haploidentical 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 0.03-0.08×10^9/dl.

Results: We started Granulocyte transfusion for three days. Granulocyte was collected from unrelated and same blood groups donors. Mean infused gran-
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).

Conclusion: AKI is an important indicator of patient outcomes after ASCT.

Aims: To investigate the incidence, causes and consequences of AKI in patients undergoing ASCT.

Methods: We retrospectively studied 154 pts, 86 (55.8%) were males with a median age of 51 years (range: 15-68), who underwent allo-HSCT in our center between May 2005 and December 2014. Patients’ baseline diseases were: acute myeloblastic leukaemia (24%), multiple myeloma (22.7%), non Hodgkin lymphoma (11.7%), acute lymphoblastic leukaemia (11%), myelodysplastic syndrome (9.1%), chronic lymphocytic leukaemia (5.2%), Hodgkin lymphoma (3.9%), aplastic anemia (3.9%), myelofibrosis (3.9%), chronic myeloid leukaemia (1.3%), Waldenström macroglobulinaemia (1.3%) and others (1.9%). Eighty (51.9%) pts received cells from matched siblings, seventy (45.5%) for unrelated donors and the remnant (3%) for HLA-mismatched donors. Twenty four (43%) pts received RIC regimens. Stem cell source were: peripheral blood (n=86), bone marrow (n=63) and umbilical cord (n=5). Median and maximum follow-up were 31 and 228 months, respectively.

Figure 1. Results: After allografting, 57.1% pts had complications, the most frequent were: infections (45.5%), followed by nephrotoxicity (25.3%), hepatotoxicity (12.3%), pulmonary toxicities (9.7%) and cardiotoxicity (3.9%). Eighty-two percent of pts were positive for aGVHD and 20.8% for cGVHD. The incidence of aGVHD was 92.3% in patients with high risk, 91.2% in patients with intermediate risk and 85.7% in patients with low risk. Mean duration of aGVHD was 3.4 months (range 0.1-24.7 months) and of cGVHD was 2.4 months (range 0.1-15.7 months). The cumulative incidence probability of aGVHD grades II to IV was 49.5%, 56.2% and 62.9% at 1, 2 and 3 months respectively. The cumulative incidence probability of cGVHD was 37.8%, 44.5% and 51.2% at 1, 2 and 3 months respectively.

Discussion: The incidence of aGVHD and cGVHD in our study is in line with previous studies. The cumulative incidence probability of aGVHD grades II to IV was 49.5%, 56.2% and 62.9% at 1, 2 and 3 months respectively. However, the cumulative incidence probability of cGVHD was 37.8%, 44.5% and 51.2% at 1, 2 and 3 months respectively.

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Conclusion: The incidence of aGVHD and cGVHD in our study is in line with previous studies.
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%) patients relapsed. Neither PAM nor HCT-CI were good predictors for relapse. However, HCT-CI was not good predicting complications, GVHD, NRM or relapse.

**Summary/Conclusions:** In our series of pts, risk-groups based on PAM score provided much better discrimination of post-HSCT complications, aGVHD (II-IV) and NRM than HCT-CI model. None of the indexes were acceptable predictors of relapse. Furthermore, correlation between both indexes was poor.

E1531

**ROLE AND TIMING OF HEMATOPOIETIC CELL TRANSPLANTATION FOR HIGH-RISK PERIPHERAL T-CELL LYMPHOMAS**

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**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 autoHCT 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.

**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 DEFIBROTIDE: POST-HAC 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 (44.5 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 59.8%, 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 AEAs (TRAEs). The TRAEs in ≥2% of patients were pulmonary hemorrhage (4.6%), gastrointestinal hemorrhage (3.0%), epistaxis (2.3%), and hypertension (2.0%).

**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:** Elevated bilirubin is a well-known marker of disease burden and in some contexts an independent predictor of survival in patients with PTCL. However, its role in the setting of chemotherapy consolidation following hematopoietic stem cell transplantation for PTCL is unknown. In this analysis, we examined the impact of elevated bilirubin on post-HSCT survival in a prospective, randomized trial of intensified chemotherapy consolidation following autologous or allogeneic hematopoietic stem cell transplantation in patients with PTCL in remission or primary refractory disease.
Cancellations for donor-related reasons and the following factors: donor sex, were extracted from BBMR databases and donor notes and were analysed retrospectively by TCs, donor reason or mixed reasons. Within donor reasons we distinguished 3 categories: donor medical reasons, donor withdrawal on personal grounds and inability to contact the donor. We examined associations between cancellations for donor-related reasons and the following factors: donor sex, age at time of donation/cancellation, time on the register and donor reliability score. The reliability score relates to blood donation and runs from 1 (best) to 5 (worst), increasing if a donor fails to attend appointments for blood donation.

**Results:** A BBMR final/bacup donor was selected for 3184 stem cell or lymphocyte collections. 82% of the requests (n=2631) were completed. Out of the 571 (18%) cancelled cases the reason for cancellation was not available for 5 cases. Overall, 6% more than half of the cancellations (248/3322, 57%) were caused by TCs mainly due to patient death, deterioration or alternative donor choice. Donor reasons accounted for 38% of cancellations (n=216, 6.8% of requested donors), of which 69% (n=148) happened for medical reasons, 27% (n=59) for donor pull-out on personal grounds and 4% (n=9) due to uncontactable donors. The medical reasons for withdrawal were varied but the most frequent health issues were obesity and cardiovascular disease. Analyses of factors affecting donor reasons showed that donor sex and time on the register were not associated with donor fitness or withdrawal rate. Age had no impact on donor pull-out, but it was significantly associated with medical eligibility and donors who were older than 50. Donor pull-out showed a significant association with blood donor reliability score (p=0.029, score 5 vs others). In 48 cases (8%) there were mixed reasons where TCs had other donor options and pursued them because of issues such as donor availability for ideal dates or CMV mismatches.

**Summary/Conclusions:** In our registry patient-related issues accounted for more than half of cancellations at a late stage in the stem cell donor pathway. Cancellations for donor reasons were unusual (6.8% of requested donors), which figure compares favourably with international data (12.4% of requested donors, WMDA Annual Report 2015). This is likely due to the fact that most BBMR donors are regular blood donors: few donors withdrew for personal reasons.

**E1535**

**POLIMORPHISM IN TGFβ1 GENE PREDISPOSES TO RELAPSE AND DEVELOPMENT OF ACUTE GRAFT-VERSUS-HOST DISEASE GRADES III-IV**

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**Background:** Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the most effective treatment option for certain hematological malignancies. Cytokines play a well established role in the mechanism of acute GvHD (aGvHD), which is one of the most significant complications after allogeneic HSCT. TGFB1 gene polymorphism and HSCT outcome. However, in patients whose unrelated donors carried homozygous TGFB1 -III-IV donors, the TT genotype seemed to be associated with a higher risk of aGvHD, which is one of the most significant complications after allogeneic HSCT. The polymorphism in the TGFB1 gene might influence the development of aGvHD.

**Aims:** The aim of this study was to investigate the role of TGFB1 -III-IV polymorphism in the outcome of HSCT.

**Methods:** We examined the association of recipient and donor TGFB1 -III-IV allele frequencies. TGFB1 -III-IV polymorphism was determined by LightCycler 480II melting curve analysis (LightCycler 480II, Roche Diagnostics). The analysis was performed in a cohort of 419 adult patients who underwent 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 -III-IV polymorphism, the SNP rs1800469 genotype was determined by LightCycler melting curve analysis (LightCycler 480II, Roche Diagnostics) was performed.

**Results:** We did not find any association between recipients' TGFB1 -III-IV polymorphism and HSCT outcome. However, in patients whose unrelated donors carried homozygous TGFB1 -III-IV donors, the TT genotype showed a higher risk of aGvHD, which is one of the most significant complications after allogeneic HSCT.

**Summary/Conclusions:** Our findings suggest that TGFB1 -III-IV polymorphism in HSCT donors might influence the development of aGvHD in unrelated and the relapse rate in related HSCT.
**E1536**

**EARLY AND LATE LOSS OF PROTECTIVE ANTIBODY LEVELS AGAINST MEASLES, MUMPS AND RUBELLA IN PATIENTS GIVEN ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION**

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**Background:** Live-vaccines should be avoided in the early period following allogeneic hematopoietic cell transplantation (HCT), due to a possible uncontrollable proliferation of the attenuated strains. The post HCT immune system is severely compromised by pharmacological immunosuppression and disruption of lymphoid tissues by conditioning and donor T cell alloreactivity. Patients frequently lost their antibody-based immunity against measles, mumps, and rubella after receiving allogeneic HCT.

**Aims:** Here, we studied the dynamics of antibody (AB) titers against measles, mumps, and rubella post-HCT.

**Methods:** We retrospectively analyzed serial AB titers in 240 patients who underwent allogeneic HCT from related und 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 fastest 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.

**Figure 1.**

**Summary/Conclusions:** We found a marked decline of AB titers post-HCT with loss of protection in a substantial proportion of patients. Surprisingly, BM grafts did not provide better AB protection post-HCT, despite their higher content of (donor) plasma cells. Together with the observations that (i) patients with lymphoid malignancies (who have received (B-) lymphocyte targeted therapies prior to HCT) had lower AB levels, while (ii) those given reduced intensity conditioning have a higher percentage of protective AB levels in the first years, our data suggest, that residual host plasma cells significantly contribute to AB production during the first years post-HCT. In opposite, the loss of protective AB levels in later years after transplantation was independent of the toxicity of the conditioning regime and may be a effect of weakening signaling for host plasma cells or late donor alloreactivity.

**E1537**

**MICA AND NKG2D POLYMORPHISMS HAVE A SIGNIFICANT IMPACT ON GRAFT VERSUS HOST DISEASE AFTER HLA-MATCHED HEMATOPOIETIC STEM CELL TRANSPLANTATION.**

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**Background:** MICA (MHC class I polymptide-related sequence A) is a highly polymorphic gene closely linked to the HLA-B locus. It encodes a cell stress inducible glycoprotein, which mediates an activatory signal towards the NKG2D receptor expressed on NK-cells, CD8+ T-cells and NKT-cells. MICA polymorphisms have been shown to influence NKG2D signaling. Indeed, a methionine to valine change at position 129 in exon 3 categorized the MICA alleles into strong (MICA-129 met) and weak (MICA-129 val) binders of NKG2D receptor. 5 repetitions of OCT with 1 additional nucleotide insertion (G) in exon 5 designated the MICA A5.1 alleles with a premature stop codon. Moreover, NKG2D polymorphisms identified alleles associated with a low (NKCS C/C and NK4C C/C) or high cytotoxic activity (NKCS G/G and NK4C T/T).

**Aims:** In this study, we hypothesized that polymorphisms at the MICA and NKG2D loci are associated with adverse outcomes in HSCT.

**Methods:** Here, we evaluated whether recipient MICA and donor NKG2D polymorphisms (respectively MICA-129, MICA A5.1 and NKCS, NK4C) could influence the incidence of acute and chronic graft versus-host disease (GVH), overall survival (OS) and relapse free survival (RFS) on 124 patients undergoing allogeneic hematopoietic stem cell transplantation using an HLA-matched donor (10/10).

**Results:** In an univariate model, recipient MICA A5.1 heterozygosity (p=0.030) and donor NK4C C/C polymorphism (p=0.013) are associated with the increase of incidence of acute GVH (grade I to IV). Recipient MICA A5.1 heterozygosity is also associated with chronic GVH (p=0.04) while Recipient MICA-129 val/val tends to be a risk factor of chronic GVH without being statistically significant. These polymorphisms have no significant impact on OS and RFS in our study (median of follow up=15 months; range 0.2-49 months).

**Summary/Conclusions:** Our data suggest that a MICA or NKG2D low activity status can be related to an increase of acute GVH according to a mechanism that remains to be elucidated, maybe by a low cytotoxic activity on recipient dendritic cells.

**Table 1.**

**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 FluBu regimen, which is used as standard (ST) for older patients at our center ST cohort.

**Aims:** To assess its impact on survival, we compared the outcomes of older patients treated with the TS Bu (TS cohort) and the reduced intensity conditioning with FluBu regimen, which is used as standard (RIC cohort) for older patients at our center.

**Methods:** Patients in the TS cohort received Flu 80mg/m2 on day -13 and -12 and Flu 40mg/m2/d followed by IV Bu on day -6 to -3, dose adjusted to achieve a total Bu course AUC of 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 adjusted to achieve AUC of 16,000µmol/min. Patients with AML or MDS were eligible for the study if they had adequate organ function, had matched related or unrelated donor and were treated between Jan 2012 and Sep 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 olderpatients with AML/MDS.
HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH DEPLETION OF TCR αβ (+) IN CHILDREN: ERCIYES PEDIATRIC BMT CENTER

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Background: Recently, haploidentical hematopoietic stem cell transplantation (HSCT) possess 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 five 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 were performed. Transplantation-related mortality (TRM) was 13.7%. The regimen included ATG, Fluudarabine, Thiotepa, Melphalan. Mycophenolate mofetil (MMF) was given as GvHD prophylaxis if the graft contained <5 x10^5/kg TcR αβ.

Results: The mean of collected CD34 cells were 18.60 (range 3.98-43.66) x 10^6/kg. The graft had a purity of 99.9% TCRαβ depletion with a median of 0.257 (range 0.003 to 1.47) x 10^5 TCRαβ cells. The median engraftment days for myeloid and platelet were both 12th day of HSCT (range 7 to 28, 9 to 33 day) respectively. Grade II skin GvHD was detected in 8 patients, and treated with steroids without any further complications. However grade III, and grade IV gastrointestinal GvHD were observed in three patients. Although the patients with gastrointestinal GvHD were treated with steroid, budsedon, cyclosporine, MSC; one patient did not respond and died. MMF was given as GvHD prophylaxis in 36 patients and 15 patients did not receive any immune suppressive drug. The mean day of discharge was 34th day of HSCT. The long term follow up including immunological reconstructions were performed in 18 patients. The analysis of the immune reconstitution of the patients transplanted in haploidentical HSCT group showed a rapid immune reconstitution for CD3+ T cells 732 (range 126-2432)/mm3; for CD4+ helper T cells 92 (range 1–419)/mm3; CD8+ cytotoxic cells 310 (range 95-2235)/mm3 at 28th day of HSCT. Ten out nine patients are currently alive, with a median follow up of 22 months (range 1 to 49 months). Overall survival was 65.9% in this group.

Summary/Conclusions: Our primary results underline that haploidentical HSCT with depletion of TcR αβ (+) can be an option in experienced center in countries which unrelated donor programs are not satisfactory, as in Turkey. The availability of a haploidentical donor in most families is a potential advantage. Moreover probably more potent graft-versus tumor effect can be induced with haploidentical HSCT.

SECONDARY MYELODYSPLASTIC SYNDROME AND/OR ACUTE LEUKEMIA INCIDENCE AFTER AUTOLOGOUS TRANSPLANTATION FOR LYMPHOMA PATIENTS IS CONNECTED WITH DECREASE OF HEMATOPOIETIC RESERVE

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2Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Background: Secondary myelodysplastic syndrome and acute myelogenous leukemia (sMDS/AML) is one of the most important long term complication of high dose therapy (HDS) with autologous stem cell transplantation (ASCT). The factors usually described to be associated with sMDS/AML development are pretreatment, HDS itself, radiotherapy, age and recently the evidence of TP53 mutations (Wong, Nature 2015) or clonal hematopoiesis (Gibson, JCO 2016) are key factors for ASCT.

Aims: The aim of the study was to analyse the incidence and risk factors for sMDS/AML after HDT and ASCT for lymphoma.

Methods: Patients who underwent HDT with ASCT for lymphoma in one centre since 12/1993 till 7/2016 were analysed. Pretreatment characteristic, graft quality, engraftment characteristics were included into analysis. Patients were censored at the time of death or allogeneic stem cell transplant. Pearson, Kaplan Maier, log-rank and cox regression tests were used.

Results: Altogether 728 pts underwent ASCT for lymphoma in given time period. 55% of pts consists out of 77% B-NHL (n=586), 6% T-NHL (n=43) and 16% HL (n=119), 58% were men, age median at the transplant was 49 years (18-71). The median of previous lines was 2 (1-9). The stem cell collection was performed after chemotherapy and G-CSF mobilization in most cases, 19 pts were mobilized by G-CSF only and bone marrow only was used in 4 pts. The targeted CD34+ dose was 2 x10^6/kg. The median number of apheresis was 2 (1-12). At the time of ASCT 90.6% of patients had chemosensitive disease (51.1% CR) and 9.4% were transplanted for chemoresistant disease. Tandem HDT and ASCT was used in 36 pts, BEAM was the most frequent HDT regimen.

Conclusions: The aim of the study was to analyse the incidence and risk factors for sMDS/AML after HDT and ASCT for lymphoma.

E1540 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 thrombo-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-genic transplant recipients between October 2008 and November 2016. All cases had non-immune hemolytic anemia with high LDH, low haptoglobin and negative Coombs test, >2 schistocytes per high-power field and thrombocytopenia (<50x10^9/L or <50% of normal baseline). Cases without signs of renal or non-thrombotic complications were excluded. Eculizumab therapy was given to all patients.

Results: We identified 17 TA-TMA episodes treated with defibrotide in 16 allo-genic 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). Covariant risk factors at the time of TA-TMA onset were: calcineurin inhibitor treatment in all cases (13 cyclosporin, 4 tacrolimus), acute GVHD grade III/IV in 8 cases, 3 CMV reactivations and 2 severe fungal (1 pulmonary aspergillosis, 1 Scedosporium Prolificans septicaemia) or bacterial (1 E Coli septis) 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
Summary/Conclusions: TA-TMA is a severe endothelial dysfunction syndrome for which, beyond the complement inhibitor eculizumab, treatment is limited, as beyond the complement inhibitor eculizumab, treatment is. This study assessed the impact of HSCT-CI on transplant outcomes in a cohort of patients with aplastic anemia and hematopoietic cell transplantation. Here, we provide encouraging evidence suggesting that defibrotide, as monotherapy or in combination with other agents, may be associated with higher HCT-CI score were associated with this improved outcome. However, this study was not validated in a cohort of patients with any of the hematopoietic malignancies. Aims: We performed multi-center retrospective study to validate the prognostic impact of HSCT-CI on transplant outcomes in a cohort of aplastic anemia patients undergoing allo-HSCT.

Methods: This study applied the HCT-CI to 140 patients with severe aplastic anemia (SAA) who underwent allogeneic HCT at the Asan Medical Center, Seoul, and Haeundae Paik Hospital, Busan, Korea between April 1995 and March 2013. Required data were retrieved from Asan medical center and Haeundae Paik Hospital BMT Registry Database. We stratified the patients based on comorbidities, as assessed by HCT-CI. Post-transplant outcomes were evaluated in terms of overall survival (OS) and event-free survival (EFS).

Results: The median age of including patients was 31 year-old (range, 31-61 year-old) and male was 81 patients (58%). HCT-CI score was 0 in 92 patients (65.0%), 1-2 in 34 (24.3%), and ≥3 in 14 (10.2%). The most prevalent comorbidity captured by the HCT-CI was infection (n=20, 14%) followed by moderate/severe hepatic comorbidity (n=17, 10%). During a median survival post-HCT follow-up period of 45.5 months (range, 4-178.4 months), 32 patients (24%) died and 20 (14%) experienced primary or secondary graft failure. The 10-year probability of OS and EFS was 73.4% and 68.3%, respectively. OS and EFS was significantly different according to HCT-CI score; the OS for HCT-CI 0, 1-2, and ≥3 at 4 years was 81.4%, 64.6%, and 60.6%, respectively (P<0.007). The EFS for HCT-CI 0, 1-2, and ≥3 at 4 years was 76.5%, 60.0%, and 56.3%, respectively (P=0.019). Multivariate analysis after adjustment for other variables demonstrated that higher HCT-CI score were associated with increased OS and EFS as judged by increasing hazard ratio compared to patients with HCT-CI score of 0 (Table 1).
plant-related toxicity. The median hospitalization time (20, range 14-70 vs 20, range 14-53, p=0.72) and the consecutive number of re-admissions after discharge (27% vs 35%, p=0.35) were also similar between Neupogen® and Nivestim® groups. In the group of HEALTHY DONORS, 95 were mobilized with Neupogen® and 122 with Nivestim®. Donor characteristics were equivalent between groups, and no severe adverse events were registered in any of them. Mean of CD34+ cells collected/kg of recipient body weight was 7.62±10^6 SD=3.45±10^6 for Nivestim® vs 6.26±10^6 SD=2.71±10^6 Neupogen®(p=0.002), but the minimal target cell dose (2x10^6/kg) was collected in all donors. 8.5% of donors mobilized with Nivestim® failed to achieve the optimal cell dose (4x10^6/kg) compared with 13% in the Neupogen® group (p=0.25). All recipients were successfully transplanted. All donors for haploidentical transplants (N=25) were mobilized with Nivestim®; none with Neupogen®. There were no other transplant differences. Platelet and neutrophil engraftment were comparable between the two groups, as well as transfusion requirements and infectious complications after transplant. The incidence of grade 1 to 4 acute graft-versus-host disease was not different (Nivestim®:65.5% vs Neupogen®: 67.7%; p=0.7). The hospitalization period was similar in Neupogen® and Nivestim® groups, (30 days, range 16-102; 30 days, 16-136, respectively).

Table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>main results comparison in patients who underwent autologous stem cell transplantation</th>
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<tr>
<td><strong>Results</strong></td>
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<tr>
<td>CD34+cells/L in peripheral blood, median (range)</td>
<td>29.07 (11.9-116)</td>
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<tr>
<td>Total platelet nadir %</td>
<td>8.26 (4.4-21)</td>
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<tr>
<td>Total red blood cell nadir %</td>
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<td>Neutrophil nadir, %</td>
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<td>Haemoglobin nadir, g/dL</td>
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<td>Neupogen® vs Nivestim® vs P value</td>
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Summary/Conclusions: Although prospective data are still required, our study suggests 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 co-morbidities than younger donors and careful assessment of their suitability to donate is required.

Aims: To analyse 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 simple template 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 ineligible during work-up, and finally 85 donors donated PBSCs to their relatives (36% of all 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 regular medications. Two thirds (67%) suffered from at least one significant comorbidity (25% hypertension, 24% back problems, 16% asthma, 9% cardiovascular conditions, 9% diabetes mellitus, 8% autoimmune disease). The presence of comorbidities was significantly associated with age (p=0.033), 59% travelled abroad, of whom 14% visited a malarial area within a year of donation. Based on donors’ history or examination findings, 47% needed extra blood tests on top of the mandatory tests before the clearance, including malaria (31%) and haemoglobinopathy screening (13%). Donors underwent 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. For clinical data collection donors’ notes were reviewed and analysed retrospectively.

E1545

LONG-TERM RESULTS OF DONOR LYMPHOCYTE INFUSIONS IN RELAPSED AND MIXED CHIMERISM PATIENTS AFTER ALLOGENIC STEM CELLS TRANSPLANTATION

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Background: Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for patients with hematological malignancies. However, relapse remains the major cause of treatment failure after allo-HSCT. Mixed chimerism (MC) can induce immunologic tolerance and lead to relapse. One of the most effective approaches to treat these patients is donor lymphocyte infusion (DLI) with or without chemotherapy.

Aims: To analyze long-term results DLI in early posttransplant MC and in relapsed patients after allo-HSCT.

Methods: The study included 61 patients of whom DLI with interleukin 2 (IL-2) was administered at the National research center for Hematology from 2011 till 2016. DLI with IL-2 was administered for patients with MC, more than 10-15% recipient DNA (n=26). A median age was 33 years old (19-54 years). Eight were males, 20 – females. There were AML (n=17), ALL (n=4), MDS (n=2), CML/MPN (n=3). Before allo-HSCT complete remission had in 20 patients and 6 had relapse/progression disease. Patients received allo-HSCT from related (n=20) or unrelated (n=6) donor. The intensity of conditioning was mainly reduced intensity (n=15) rather than myeloablative conditioning (n=11). Bone marrow (BM) as a graft source was used in 20, PBSC – 6. DLI was started at low dose 1*10^7CD3+ per kg. Every following dose of infusion CD3+ increased until 5*10^7CD3+ per kg. Number of infusions depended on achievement 100% donor chimerism. Thirty five patients with relapse after allo-HSCT (AML, n=27, ALL, n=5, MDS, n=2, CML, n=1) were administered DLI with IL-2. Number DLI was 1 or 5 in different causes. Complete remission before allo-HSCT had in 25 patients and 10 had relapse/progression disease. 33 patients received chemotherapy and after chemotherapy on 7 days DLI was using an escalating dose following infusions. Two patients received DLI with IL-2 without chemotherapy. A median age was 33 years old (18-60 years). 14 were males, 21 - females. Stem cell source was BM and (PBSC) in 22 and 13 of the cases, respectively. Patients were transplanted from related (n=17) and from an unrelated donor (n=18). Condition regimen was MAC (n=7), RIC (n=28). Bone marrow as a graft source was used in 22, PBSC – 13.

Figure 1.

Results: A median follow up was 5 months (0.3-63). A median time between allo-HCST and DLI was 3 months (1.5-64). 100% donor chimerism was achieved in 17 patients with MC from 26 (65%). A median number of infusions...
was 2 (1-5). There were 5 (19%) graft failures. Acute GVHD appeared in 8 (32%), all of them grade 3; chronic GVHD occurred in 7 (27%). Patients with a MC had better overall survival 77.6% than patients with relapse after allo-HSCT (22%). Remission was achieved in 16 (48%) patients with relapses. However, 5 patients relapsed again. Acute GVHD was developed in 8 cases (22%). Nineteen patients died from relapse and 1 patient died from aGVHD in remission. Five patients survived in patients with MC and in patients with relapses it was 78.6% and 26.2%, respectively.

Summary/Conclusions: The prognosis of hematological malignancies is poor if relapse is established after allo-HSCT. DLI protocol as preventive therapy must be created for improving long-term results in high risk patients. Prevention is better than cure.

E1546

MEMORY T CELLS DONOR LYMPHOCYTE INFUSIONS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION AS A SAFE PROCEDURE TO IMPROVE T-CELL RECONSTITUTION

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Background: Hematopoietic stem cell transplantation (HSCT) is a potential curative treatment for patients with hematologic malignant diseases. Haploidentical transplantation with extensive ex vivo T cell depletion of the graft, has demonstrated to prevent graft versus host disease (GVHD), but the major disadvantage has been the development of graft failure, relapse and infections due to delayed immune reconstitution. A selective T cell depletion method that removes T 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-refractory 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 3x10^6-9x10^6/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 3-18 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 1.02x10^6/kg, starting at a dose of 0.2x10^6/kg for 10 days and the maximal dose of 2x10^6/kg were used every 21 days. The CD45RA+ cell dose was a median of 0.004x10^6/kg (range: 0.1-6x10^6/kg). All the procedures were well tolerated, neither adverse events nor GVHD were noticed. After the DLI, a progressive increase in T cells count were observed.

Summary/Conclusions: In our experience DLI enriched for CD45RO+ memory T cell is a promising and safe strategy for patients with severe viral infections and risk of relapse after haploidentical HSCT, these cells has demonstrated to trigger the CD4 and CD8 T cell reconstitution, which will help reduce risk infection with a low risk of GVHD. However further studies are needed in order to support this therapy.

E1547

FLAG REGIMEN WITH IDARUBICINE AS 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 risk after transplantation.

Aims: We have evaluated the safety and efficacy of new conditioning regimen with sequential intensive chemotherapy (FLAG-IDA) followed by conditioning of Flu-Bu(3).

Methods: The study was designed and developed in two separate transplantation centers in Rui Jin Hospital (RJH, Shanghai) and Institut Paoli-Calmettes (IPC, Marseille) respectively. A total of 47 refractory AML patients with median bone marrow blast of 30% (1-90%) and median age at diagnosis 60 (16-82) were enrolled. Thirteen patients received transplantation with mobilized peripheral blood stem cells (PBSC) from HLA-matched sibling donor while 18 and 16 with matched unrelated or haplo-identical donors. All patients received FLAG + 3-days idarubicine (12mg/m² in RJH or 10mg/m² in IPC) and then received Flu-darabine (5 days) with IV Busulfan (3-days) with a 7-day interval. The GVHD prophylaxis regimens were CsA+MMF+ATG (RJH) or post-cyclophosphamide (IPC).

Results: With a median follow-up of 8 months (1-70m), a total of 14 patients relapsed with a median time of relapse at 4.8 months (2.1-18.1) and most of the patients relapsed within 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±7.8%. In the primary multivariate analysis (including age, cycles of pre-transplantation chemotherapy, bone marrow blasts, cytogenetics and treatment center), only bone marrow blast ≥35% and age over 40 were associated with disease-free survival and relapse respectively while there was no signific-ant difference between RJH and IPC in terms of transplantation outcome in univariate and multivariate analyses.

Summary/Conclusions: Our primary data demonstrated a promising outcome with FLAG-IDA chemotherapy as debulking therapy sequential with Flu-Bu3 conditioning regimen in patients with refractory AML and clinical trial with larger patients cohort is warranted.

E1548

STUTTER PCR PRODUCTS MAY NOT INTERFERE WITH STR BASED CHIMERISM MONITORING AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Background: Chimerism analysis is one of the main methods to monitor the bone marrow engraftment or disease relapse after allogeneic bone marrow transplantation. Routine test is based on differences in the length of short tandem repeats (STR) from the donor and the recipient. However, chimerism estimation is complicated by stutter PCR peaks appearing due to irregular DNA polymerase activity. Generally, these sequences are 4 nucleotides shorter than a specific marker and may concur with a specific sequence of recipient’s DNA. The problem seems to be especially serious in case of a sex-matched sibling BMT when most of the alleles for donor and recipient are the same. One may suggest to limit the use of these markers for the cases with stutter-bands comparable with donor allele peak height. Therefore, the absence of "stutter-peaks free" markers hinders the chimerism estimation at the point of low recipient hematopoiesis output.

Aims: To identify the contribution of stutter-bands to the total amount of PCR-product and to derive universal formulas for the chimerism calculation excluding stutter percentage.

Methods: Genomic DNAs of donors and patients were isolated from bone marrow samples. Chimerism was assessed by the STR-PCR analysis (polymeric chain reaction with a panel of primers for loci of short tandem repeats) on DNA sequencer 3130 (Applied Biosystems). The fragment analysis was performed on a 3130 Genetic Analyzer. The data processing was accomplished using GeneMapper v4.0 software. Informative loci were chosen beforehand comparing pretransplantation
INTRODUCING PLERIXAFOR TO IMPROVE MOBILIZATION IN MULTIPLE MYELOMA PATIENTS WHO BEHAVE AS POOR-MOBILIZERS IS COST-EFFECTIVE CONSIDERING THE WHOLE MOBILIZATION AND TRANSPLANT PROCEDURE

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Background: Plerixafor, a CXCR4-antagonist, is efficient to improve CD34+ cell mobilization and collection in candidates for autologous transplantation who behave as poor-mobilizers. The cost of the drug is however of concern. 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 20x10⁶/L. Plerixafor was introduced or not as a result of the attending physician’s decision, reflecting progressive changes in medical practices over this transitional period. The historical and study groups were matched over four criteria: disease stage at diagnosis, age, gender and number of chemotherapy treatments received before mobilization. Two cost-effectiveness analyses (CEA) were conducted: the primary CEA looked at the criterion “collecting at least 2x10⁶ CD34+ cells”; a secondary CEA looked at the criterion “successful autologous transplant administered”. Detailed micro-costing evaluations (2015 figures) did not or did include transplantation costs for the first and second CEA respectively.

Results: The two groups were similar in terms of age, sex distribution, disease characteristics or previous treatments. 27/30 and 26/30 patients proceeded to high-dose melphalan and autologous transplantation in the study and historical groups, respectively. There was a trend to a higher number of collected CD34+ cells/kg in the historical group (8.757 vs 5.460 €, p=0.001), and proportionally higher in patients who received plerixafor as part of a remobilization treatment rather than pre-emptively (10.401 vs 8.162€ respectively). The main CEA concluded to a 3.237€ increase in costs for the same number of patients achieving a minimal target number of 2x10⁶ collected CD34+ cells/kg. The second CEA found a decrease in the cost of transplant, with 12.724€ in the study group and 12.364€ in the historical group (NS). In total, the 2.035€ increase for the complete procedure cost (22.866€ per successfully autografted patient in the study group vs 20.831€ in the historical group) was not statistically different.

Summary/Conclusions: Cost-effectiveness arguments should not be used against the administration of plerixafor in multiple myeloma patients in the European context. Future prospective researches looking at patients reported outcome criteria and of poor administration in apheresis facilities are needed.
(RBC) recovery, neutrophil and platelet engraftment, pure red cell aplasia (PRCA), acute GVHD, relapse and event-free survival (EFS).

**Methods:** We retrospectively studied allogeneic transplants performed from January 1, 2013 to December 31, 2016. We collected the baseline variables reflected in Table 1 and analyzed the incidence of HE, neutrophil and platelet engraftments, RBC recovery, PRCA (defined as anemia with transfusional requirements and reticulocytes <1% in day +80 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=11) and, in most cases, hematopoietic progenitors were obtained from mobilized peripheral blood (90.2%), 44.3% (n=59) presented some type of ABOi: major (n=26), minor (n=25) and bidirectional (n=8). The product was processed in order to prevent hemolysis in only 7 cases (red cell depletion in 4 and deplasmatization in 3). There were 23 hematolysis (18 immediate and 5 delayed) - mostly mild- events, which appeared predominantly in patients with ABO-incompatibility (92.3%), 44.3% (n=59) presented some type of ABOi: major (n=26), minor (n=25) and bidirectional (n=8). The product was processed in order to prevent hemolysis in only 7 cases (red cell depletion in 4 and deplasmatization in 3). We have not found differences either in the rate of relapse (24.8% vs 19%) 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>
<th>Age (years)</th>
<th>ABOc (n=50)</th>
<th>ABOi (n=38)</th>
<th>ABO (n=133)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>52</td>
<td>53</td>
<td>52</td>
<td>52</td>
</tr>
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<td>Female</td>
<td>52</td>
<td>52</td>
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<td>52</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** In our study ABO-mismatched transplants have shown a greater number of hemolytic events and red cell aplasia, as well as a greater delay in achieving erythrocyte recovery. However, we have not found an association with delayed neutrophil and platelet recoveries, increased acute GVHD, relapse or worse OS in the ABO incompatible group, in keeping with previous reports**, although the absence of effect might be as well be related to an insufficient study power due to low sample size.

**References**


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**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 uncertain.

**Aims:** The objective of the current study was to investigate association between expression of TLRs genes and hematopoietic recovery and rate of infections in patients treated with PBSCT.

**Figure 1.** Summary/Conclusions: Low levels of Tac blood concentration were significantly associated with the incidence of graft failure of the patient for whom Tac with an additional sMTX were used for GVHD prophylaxis. Before engraftment, frequent checks of the Tac blood concentration and maintaining the drug level should be considered for these patients.

**E1552**

**LOW BLOOD CONCENTRATION OF TACROLIMUS CAN BE A RISK OF GRAFT FAILURE AFTER BONE MARROW TRANSPLANTATION**

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**Background:** Bone marrow 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. Immuneological rejection of the graft is known as a major cause of graft failure. Graft failure may also be caused by septicemia, 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 10-20mg/ml.

**Results:** Of the 59 patients, 48 patients achieved neutrophil recovery at a median of 22 (range 13-35) days. Two patients died before engraftment from severe PIR and active infection. Nine patients (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 (MTX group) for GVHD prophylaxis (p<0.01). Combined of these factors, the patients of Tac low group and MTX group had an incidence of graft failure 40.9%, which was significantly highest than the 5.4% seen in the other patient including Tac high group and MMF group even if the patient were included of Tac low group.

**Table 1.**

<table>
<thead>
<tr>
<th>Tac Blood Concentration (ng/ml)</th>
<th>Graft Failure</th>
<th>Tac Low Group (n=26)</th>
<th>Tac High Group (n=33)</th>
<th>Tac MMF Group (n=33)</th>
<th>Tac MTX Group (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>15</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>11-20</td>
<td>12</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>21-30</td>
<td>12</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>31-40</td>
<td>10</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
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<tr>
<td>51-60</td>
<td>10</td>
<td>1</td>
<td>1</td>
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<tr>
<td>&gt;60</td>
<td>10</td>
<td>1</td>
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<td>1</td>
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</tbody>
</table>

**Summary/Conclusions:** Low levels of Tac blood concentration were significantly associated with the incidence of graft failure of the patient for whom Tac with an additional sMTX were used for GVHD prophylaxis. Before engraftment, frequent checks of the Tac blood concentration and maintaining the drug level should be considered for these patients.
Methods: The evaluation of TLRs expression genes were performed in 40 patients who underwent PBSCT. The median age of patients was 54 years (range: 25-65 years). There were 15 patients with multiple myeloma (MM), 20 patients with non-Hodgkin lymphomas (nHLs) and 5 patients with Hodgkin lymphoma (HL). Peripheral blood samples were taken before megachemotherapy with autologous stem cell transplantation and at time of hematopoietic recovery in patients with PBSCT. Relative expression of receptors was assessed by real-time PCR using inventoried TaqMan® Assays from Life Technologies/Thermo Fisher. 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 GraphPad Prism 4.0. In cases of independent quantitative variables the non-parametric test was applied. In cases of quantitative variables with the normal distribution the statistical analysis took advantage of t test for unlinked variables. In cases of variables manifesting distribution distinct from 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 (ΔCt HL 1,429,91 ± 1,0461 vs 1,7877 ± 1,4974 and ΔCt TLR9 116,85 ± 1,0470 vs 289,78 ± 2,7198 (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 (ΔCt TLR9 117,141 ± 0,870 vs 289,88 ± 2,7198 (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-diagnosis timing of initiation of defibrotide after diagnosis of VOD/SOS in HSCT patients.

Methods: In an expanded-access study, patients diagnosed with VOD/SOS (per Baltimore criteria, modified Seattle criteria or biopsy) with or without renal/pulmonary MOD after HSCT or chemotherapy received defibrotide 25 mg/kg or 2.5 mg/kg (recommended x21 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, patients with VOD/SOS versus MOD subgroup (<p=0.05).

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=0.01), except Day 14 (2.6% of patients started defibrotide after Day 14). The trend test for particular initiation days also showed a significant trend over time for higher Day +100 survival with earlier defibrotide initiation post-diagnosis in both the overall HSCT group and MOD subgroup (<p=0.01). Adverse events (AEs) and serious AEs occurred in 70.8% and 53.4% of patients, respectively. Other than VOD/SOS and MOD, the most common AE was hypotension (11.7%) and most common serious AE was respiratory failure (7.3%).

Support: Jazz Pharmaceuticals.

E1555

RED BLOOD CELL DISTRIBUTION WIDTH (RDW) AS AN ACUTE GRAFT VERSUS HOST DISEASE PREDICTOR MARKER IN ALLOGENIC STEM CELL TRANSPLANTATION

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Background: The red blood cell distribution width (RDW) is a common parameter for measuring anisocytosis in the study of anemia. Recently it has been regarded as a surrogate marker of inflammation and adverse outcome in several diseases. Acute graft-versus-host disease (GVHD) is a common complication of allogeneic hematopoietic cell transplant (allo-HSCT) which is related to inflammation in the context of damage of the host tissue and the release of inflammatory cytokines. We decided to study the utility of this potential inflammatory marker in the setting of GVHD in the allo-HSCT.

Table 1.
Aims: RDW values were evaluated at the day of infusion (RDW0), in order 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. The haploidential HSCT subgroup an increased RDW >16 was associated with acute GVHD. (p=0.044). There was no association of chronic GVHD with elevated RDW at day 0 (p=0.563). The survival analysis didn’t found an association of high RDW levels with mortality or survival (p=0.301) but a tendency to an increased survival was show between the RDW level subgroups. (figure2).

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 as 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 TRANS-PLANTATION 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 unfavoured 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 BCNU group. 22.72% of the patients receiving BeEAM in SD or in diseases progression achieved CR versus 10.86% respectively for the BEAM group. The mean time to hematological recovery for neutrophils was 11.27 days (BeEAM) versus 10.24 days (BEAM) and 12.64 days (BeEAM) versus 11.12 days (BEAM) for platelets.

Figure 1.
Summary/Conclusions: BeEAM appears to be a non-inferior alternative conditioning regimen to the standard BEAM, it shows a trend towards higher myelotoxicity, but also a trend towards better short-term results in chemoresistant patients.

E1557
DOUBLE UMBILICAL CORD BLOOD TRANSPLANTATION IN ADULTS: CORRELATION OF ALLELE-LEVEL HLA MATCHING WITH OUTCOME AND WHICH CORD BLOOD UNIT WILL BECOME DOMINANT

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Background: Umbilical cord blood (UCB) has been used for alternative donor transplantation for the past 3 decades. Graft failure is not uncommon due to higher degrees of histo-incompatibility between recipient and UCB units and fewer hematopoietic precursors in the product. To improve engraftment rates, especially in larger (i.e. adult) patient (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 ≥6/8 match at A, B (serologically) and DRB1 (by HRT). Combined TNC count for the units had to be ≥3×10^9/kg recipient weight. Conditioning was Fludarabine 40mg/m² ×4 and TBI 150 cGy ×8; GVHD prophylaxis was Tacrolimus/Mycophenolate. Pts received G-CSF 300 mcg s.c. daily from day +1. Outcomes were compared using Fisher’s exact test.

Results: Between 06/09 and 09/16, 31 pts underwent DUCBT - 11 males, 20 females with median age 50 years (range 19-59). Diagnosis was acute myeloid leukemia (AML; n=12), acute lymphoid/mixed phenotype leukemia (n=7), chronic lymphoproliferative disease (n=5), MDS (n=4) or other (n=3). All 31 pts recovered ANC>0.5×10^9/L at median of 20 days (range 14-72). Platelet count reached >20×10^9/L in 26/31 pts at median of 38 days (range 24-188). Acute GVHD developed in 26/31 pts (84%) and chronic GVHD in 17 of the 26 pts (65%) that survived to day +100. Seventeen pts (55%) remain alive, in contin-

Figure 1.
Summary/Conclusions: BeEAM appears to be a non-inferior alternative conditioning regimen to the standard BEAM, it shows a trend towards higher myelotoxicity, but also a trend towards better short-term results in chemoresistant patients.
uous remission at median follow-up of 3 years (range 0.5-7.0). Ten pts (32%) experienced non-relapse mortality from GVHD (5 pts), infection (4 pts) or unknown cause (1 pt). Four pts (13%) had relapsed at 3, 5, 10 and 12 months. Outcomes for pts when the best cord unit match was 0-2 antigen-mismatched (Ag-M/M) were superior (8/12 alive and well) to those pts when the best unit was ≥3 Ag-M/M (3/9 alive and well; p=0.20). Unexpectedly, 6/9 pts whose best unit was ≥4 Ag-M/M 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>
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<th>HLA Match</th>
<th>Higher TNC</th>
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<th>Lower TNC</th>
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<tr>
<td>Better (&lt;2%)</td>
<td>34 (34%)</td>
<td>10 (10%)</td>
<td>1 (1%)</td>
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<td>Same (2-3)</td>
<td>6 (6%)</td>
<td>20 (20%)</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Worse (&gt;3)</td>
<td>1 (1%)</td>
<td>5 (5%)</td>
<td>0 (0%)</td>
</tr>
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</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-M/M) may have better outcomes than pts receiving 3 Ag-M/M units although successful outcomes can be seen even with a high degree (≥4 Ag-M/M) of HLA incompatibility.

### Figure 1. Short-term reconstitution in BM and PBSC recipients with and without Post-HSCT-Cy.

**CLINICAL ANALYSIS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR 46 ACTIVE RELAPSED AND REFRACTORY ACUTE PEDIATRIC LEUKEMIA**

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**Background:** Given the dismal prognosis for relapsed and refractory (R/R) acute leukemia, many physicians discourage offering hematopoietic stem cell transplantation (HSCT) to adults with bone marrow (BM) blasts over 25%. Therapeutic recommendations for pediatric subjects with a similar situation are not available.

**Aims:** With no significant alternative managing options for these patients, more data are required to make an informed and patient tailored decision.

**Methods:** We retrospectively analyzed the preliminary outcome of 46 active R/R acute leukemia patients transplanted between 2012 and 2016. Median age at HSCT was 13 years. Active R/R disease was all confirmed by cytogenetics/molecular genetics and aggressive clinical course. Median bone marrow blasts was 46.4% (5-99%). Of note, 27 patients had over 50% blasts in BM. The earliest 13 transplants were conditioned with conventional BuCy or TBI/Cy regimen, thereafter, all received intensified conditioning includingFLAG/TBI (N=21), FLAG/BuCy (N=2) and CLAG/BuCy (N=10). Immuno-suppressive agents withdrawal started since day 30 if no acute GVHD occurred. Variety of post-HSCT intervention including donor lymphocytes infusion and intratejene-2 injection were performed to reduce relapse. Median follow-up of the whole cohort is 19 months (3-53 months).

**Results:** Forty-five (97.8%) achieved CR following HSCT. One died of infection before engraftment. All 3 death occurred before 90 day due to relapse. Transplant-related mortality at 1 year was 15.2%. Acute GVHD incidence was 49.3% (grade III 20.4%), chronic 59.5%. Relapse was the major cause of treatment failure and occurred in 28.3% of patients at a median of 1 year post HSCT. Two-year overall survival and leukemia-free survival were 44.8±9.5% and 44.6±8.9%, respectively. Survival of AML patients was superior to those of ALL. Refractory disease was equivalent to those with relapsed refractory AML which not seen in ALL. Blast percentage in BM pre-HSCT, TBI based conditioning and chronic GVHD proved to be favorable prognostic features.

**Summary/Conclusions:** This may validate decision making on if this special group of patients should receive HSCT as salvage treatment.

### E1559

**POST-TRANSPLANT HIGH-DOSE CYCLOPHOSPHAMIDE AFFECT T-CELL RECONSTITUTION IN BONE MARROW, BUT NOT IN PERIPHERAL BLOOD STEM CELLS RECIPIENTS**

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**Background:** Hematopoietic stem cell transplantation (HSCT) it the only curative therapy for many patients with hematologic malignancies. Occurrence of complications and mortality after allo HSCT still high and it’s strongly associated with immune reconstitution. Despite the wide-spread of Post-Transplant High-Dose Cyclophosphamide (PTCy) immune reconstitution and immunological safety of this method is still poorly understood.

**Aims:** Evaluated immune reconstitution profile in patients who received HSCT with and without PTCy.

**Methods:** 62 patients who underwent alloengenic PBSCST in our institution were analyzed in 2 groups; patients with PTCy (n=28) and without PTCy (n=34). The total cohort had 22 males and 40 females, and had median age of 33 years (range 18-81). All patients had hematological malignancy. 21 patients underwent myeloablative conditioning and 41 patients were non-myeloablative. In 41 patients received bone marrow transplant. The GVHD prophylaxis consisted of a combinations of ATG-PTCy-CsA-MMF (n=10), ATG-PTCy (n=5), Mono- PTCy (n=1), ATG-CsA-MMF-MTX (n=20), CsA-MTX (n=2), ATG-CsA-MTX (n=2), CsA-MMF-MTX (n=1). In 21 patients received PBSCT. The GVHD prophylaxis consisted of a combinations of ATG-PTCy-CsA-MMF (n=4), ATG-PTCy (n=8), ATG-CsA-MMF-MTX (n=8), ATG-CsA-MTX (n=1). 21 patients had progression disease before transplantation, this could affect the results. Immune reconstitution profile was tested via serial flow cytometry analysis of peripheral blood on day +14, +30, +60 and +90 after allo-HSCT. Anti-CD3 FITC, anti-CD16PE, anti-CD56 Per-Cp-Cy7.5, anti-CD4 PE-CL5.9, anti-CD19 APC, anti-CD8 APC-Cy7, anti-CD62L FITC (BD Biosciences, USA); anti-CD14PE, anti-CD16PE, anti-HLA-DR APC (eBiosciences, USA) were used to defined white blood cells subsets.

**Results:** In a bone marrow recipients the number of CD4+ cells was significantly lower when using PTCy (see Figure 1). On day 14 CD4+ cells counts for bone marrow recipients was 42.6±29.99; on day 30 - 114.2±42.36; on day 60 – 140.8±52.53; on day 90 - 126.8±26.12. On day 14 CD4+ cells counts for PBSC recipients for treatment failure. There is no standard therapy for relapse after SCT.

**Summary/Conclusions:** Lymphocyte recovery was impaired for the PTCy groups in the immediate post-HSCT period but quickly recovered. The mechanism of immunosuppression induced using PTCy on the +3+4 day is 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 ALLOGENEIC STEM CELL TRANSPLANTATION FOR AML IN FIRST CR: SINGLE CENTER EXPERIENCE**

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**Background:** Allogeneic stem-cell transplantation (SCT) is a curative therapy for patients with AML but disease relapse continues to be the most common reason for treatment failure. There is no standard therapy for relapse after SCT and treatment results are very poor. Treatment options range from supportive care through chemotherapy and donor lymphocyte infusion (DLI) up to a second SCT from the same or a different donor.

**Aims:** 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 adults with AML in first CR underwent allo-SCT. We identified 36/130 patients (27%) who had relapsed and proceeded to review the management and outcomes of these patients; the incidence of relapse was 20% and 54% after myeloablative and reduced intensity conditioning, respectively. The median time to disease relapse after allo-SCT was 11 months (range 5-48). 15/36 (41%) of relapsed patients suffered aGVHD grade II-IV or extensive cGVHD. At time of relapse 15/36 (41%) patients were still taking immunosuppressive treatment, which was immediately suspended.
Results: The patients were subdivided into three groups according to the salvage treatment received: palliative/supportive care (PSC group, n=9, 25%), intensive chemotherapy alone (ICH 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, ICH and IT groups, respectively. In the ICH group, 3 patients (16%) had a second CR and 4 (22%) died during induction chemotherapy. In the IT group, 6 (66%) pts achieved a second CR after chemotherapy and DLI/second allo-SCT and 3 (34%) died of treatment toxicity. In the whole patients sample, median overall survival (OS) was 7 months (range 2-74), being 4, 5, 13 months in the PSC, ICH and IT group, respectively. Estimated 1-year and 2-years overall survival was 10%, 15%, 40% and 0%, 0%, 12% in the PSC, ICH and IT groups, respectively. In our experience, 3 independent factors for a longer OS after chemotherapy and immunotherapy have been identified: the absence of previous acute or chronic GvHD (HR=2.7,p<0.001), a longer interval between the allo-SCT and relapse than 12 months (HR=1.2, p<0.005) and age less than 40 years (HR=1.3, p<0.005).

Summary/Conclusions: This study shows that salvage chemotherapy (with DLI or second allo-SCT) provides the best results and should be offered, whenever possible, to patients with AML who relapse after allo-SCT performed in first CR. Patients undergoing chemotherapy alone had a poorer outcome. Our results underscore 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 CHEMOREFRACTORY HODGKIN LYMPHOMAS: A RETROSPECTIVE MULTICENTER EXPERIENCE OF THE RETE EMATOLOGICA PUGLIESE (REP)

V. Pavone1,2, F. Gaudio2, G. Specchia2, P. Galeni1, N. Cascavilla4, P. Mazza1,3,4, J.M. Moraleda-Jiménez1
1Haematology, Panico Hospital, Tricase, 2Haematology, Policlinico Hospital, Bari, 3Hematology, Mazzoni Hospital, Ascoli Piceno, 4Hematology, “Casa Sollievo della Sofferenza” Hospital, San Giovanni Rotondo (FG), 1Haematology, Moscati Hospital, Taranto, Italy

Background: Second-line salvage high-dose chemotherapy and autologous stem cell transplantation (SCT) have become the standard of care for refractory chemotherapy-refractory (CR/CR-PR) HL. However, the results are to durable remission ranging from 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.

Methods: The retrospective 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 specific 1-year and 2-years overall survival was 15%, 10%, 40% and 0%, 0% in the PSC, ICH and IT group, respectively. In our experience, 3 independent factors for a longer OS after chemotherapy and immunotherapy have been identified: the absence of previous acute or chronic GvHD (HR=2.7,p<0.001), a longer interval between the allo-SCT and relapse than 12 months (HR=1.2, p<0.005) and age less than 40 years (HR=1.3, p<0.005).

Summary/Conclusions: This study shows that salvage chemotherapy (with DLI or second allo-SCT) provides the best results and should be offered, whenever possible, to patients with AML who relapse after allo-SCT performed in first CR. Patients undergoing chemotherapy alone had a poorer outcome. Our results underscore the need to schedule a prospective protocol combining cytoreductive treatments and immunotherapy in patients in AML relapsing after allo-SCT.

E1562
RESULTS OF THE IMPLEMENTATION OF CRYOTHERAPY IN PROTOCOLS OF ORAL MUCOSITIS PROPHYLAXIS IN PATIENTS SUBJECT TO A TRASPANT OF HEMATOPOYETIC PROGENITORS. EXPERIENCE OF ONE CENTER

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Background: Oral mucositis (OM) is one of the main complication during stem cell transplantation (SCT). It has an incidence varies between 47-100%. Numerous prevention strategies have been studied. However, the recommendations of the international guidelines have low evidence to back them up. Cryotherapy is used to reduce OM in conditions that use Melphalan. In our center, we have the cryotherapy implemented in our OM prevention protocol since 2012.

Aims: The main aim is to compare the results in terms of incidence and severity of OM (measured according to World Health Organization scale) in patients in whom cryotherapy was applied and in whom it was not applied as well as the necessity of using morphine and parenteral nutrition. The secondary endpoint is to analyze the occurrence and duration of fever and documentation of infection.

Methods: We used a cohort of patients with plasma cell dyscrasias who underwent autologous stem-cell transplant with conditioning melphalan 200, Busulphan-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). We did not collect 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 multivariable and univariable analysis. Hazard ratios and 95% were estimated with the use of logistic regression model.

Results: The baselines characteristics can be seen in table 1. All patients in both groups had OM. In the cryotherapy and non-cryotherapy groups, the distribution was respectively: grade I 20% vs 16%, grade II 40% vs 10.8%, grade III 31.4% vs 59.4% and grade IV 8.5% vs 13.5%. We observed a reduction in the incidence of severe mucositis (grade III and IV) in the group in which cryotherapy was used against the cohort in which it was not (40% vs 72.9%, p=0.005). The need for morphine was also lower in the cryotherapy cohort (54% vs 72%, p=0.149). The use of parenteral nutrition was lower in the non-cryotherapy group (8.5% vs 13.5%, p=0.7). The prevalence of fever was predominant in the cryotherapy group (51% vs 43%, p=0.48), but and infection was documented on more occasions in cryotherapy group (27% vs 81%, p=0.04). 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 non-cryotherapy group (0% vs 8%, p=0.24).

Decreased mucositis degree was associated in both univariable and multivariate analysis only with cryotherapy (p= 0.01 and p=0.0003). Hazard ratio was 0.81 (IC 95% 0.06-0.55).

Table 1.

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<th>Characteristics</th>
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<th>Non-Cryotherapy</th>
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<td>Age Median yrs</td>
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<td>OS 5-years (%)</td>
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<td>OS 10-years (%)</td>
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<td>OS 15-years (%)</td>
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<td>OS 20-years (%)</td>
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<td>OS 25-years (%)</td>
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<td>OS 35-years (%)</td>
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<td>OS 40-years (%)</td>
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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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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 systematically 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 series. We found that 4 patients (3 children, 1 adult) required a boost of CD34 selected graft from the same donor for secondary GF and poor graft function.

Table 1.

Summary/Conclusions: PGF incidence described in literature is 5-10%, we did not find any primary graft failure in our series. 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.
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 allosresponsess 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 allosresponses of APB and umbilical cord blood (UCB) T-cells after exposure to the widely used IMiD lenalidomide.

Figure 1.

Methods: We have used multi-parameter flow cytometry and gene expression profiling to perform an in-depth characterisation of the phenotypic and genotypic effects of clinically relevant concentrations of lenalidomide treatment on T-cells during allogeneic co-culture. Using GCSF-mobilised APB (GMPB), steady state APB and UCB PBMC as responder cells in allogeneic co-culture we have been able to compare the differential effect of lenalidomide on these three cell sources. Allogeneic responder cells were labelled with CFSE (carboxyfluorescein diacetate succinimidyl ester) to allow quantification of allo-proliferation. Responder T-cell subsets including naive, memory, activated, cytotoxic and regulatory were interrogated. Functional effects of lenalidomide treatment including cellular capacity to produce cytokines, degranulate and exert direct cytotoxicity was also assessed. RNA was extracted from highly purified proliferative and non-proliferative CD8+ T-cell fractions following a combination of multi-parameter flow cytometry and gene expression changes assessed by Affymetrix whole genome array and qRT-PCR.

Results: We demonstrate that lenalidomide increases net alloproliferation of APB T-cells by selectively enhancing 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 allosresponses of T-cells from different cell sources, with a potentially tolerogenic effect on UCB T-cells. These findings have important implications for the future use of IMiDs in the setting of AHSCT.

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 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.

Aims: 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 assay for marker genes analysis instead of MLR assay to investigate the relationship between gene expression profile and iTreg cell function. The data of marker genes analysis were shown in Fig. 2. 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

Figure 1.

Methods: Mouse splenocytes were prepared from mouse spleen. Human PBSC were prepared from peripheral blood (PB) of healthy donors by Ficoll-Hypaque density gradient centrifugation. All T cells were isolated by negative selection, then CD4+ naïve T cells were harvested. CD4+ naïve T cells were activated with anti-CD3/CD28 beads in the presence of IL-2, TGF-β and retinoic acid (RA) containing RPMI1640 medium. The protocol is showed in Fig. 1.

Results: Seven genes for qPCR analysis were used to identify the functional iTreg cells. We used the different proportions of iTreg cells in total naïve T cells for 7 genes expression analysis and MLR assay to investigate the relationship between gene expression profile and iTreg cell function. The gene expression analysis were shown in Fig. 2. 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 coincidene of the data in Fig.2 (Fig.3). Using the same iTreg populations, MLR assay have been investigated for 5 days. The T cell suppression percentage would be dependent on the iTreg cells proportion (Fig.4A and B). It indicated that the gene expression levels can represent the biological function of iTreg cells. It’s the better way to identify the iTreg cells. Further, we have used PBMCs for Treg cell induction, the marker genes expression analysis also showed in Fig.5. After comparing with IL-2 cultured T cells, the gene expressions revealed the difference in between iTreg cells and un-induced T cells.

Summary/Conclusions: Our study showed that MLR assay should spend 3 to 5 days for identification of the functional iTreg cells, however, the marker genes analysis took only one day for that. Besides, MLR assay is a more complicated method than qPCR analysis. Using simple analysis for human iTreg cells functional identification could save the time for clinical application and might prevent GVHD occurrence effectively.

E1568

OXIDANT-ANTIOXIDANT SYSTEM IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is one of the most widespread malignant B-cell lymphoproliferative disorders and is characterized by a clonal proliferation of atypical plasma cells in bone marrow or, less frequently, in extramedullary locations synthesizing monoclonal immunoglobulins. Currently, autologous hematopoietic stem cell transplantation (auto-HSCT) is recognized as the standard method of treatment for young patients (≤65 years old) with MM. Moreover, the best auto-HSCT results are observed in patients who have received new medication (thalidomide, bortezomib, and lenalidomide) during induction therapy and who have achieved at least a very good partial response, which leads to a significant increase in overall survival. However, studies reflecting the impact of this kind of treatment on the dynamics of oxidant-antioxidant indicators are virtually non-existent. At the same time, the possibility of treating developing diseases by prescribing medication makes the problem highly relevant.

Methods: We studied 20 patients (11 men and 9 women, mean age 49 years) who followed auto-HSCT after high-dose melphalan. The control group consisted of 50 age- and sex-matched healthy persons. The plasma levels of malonic dialdehyde and ceruloplasmin as well as activities of superoxide dismutase and catalase were measured by standard biochemical techniques. In erythrocytes, the level of non-protein thiol groups was decreased in MM (p<0.05). The results of our study have shown, that an imbalance of OS-AOS is frequently seen in MM patients, and, possibly, could influence the course of auto-HSCT.

Summary/Conclusions: The results of our study showed that auto-HSCT in patients with MM during auto-HSCT.

E1569

SURFACE RECEPTOR EXPRESSION PROFILE DEFINES ALLOGREACTIVE DONOR CD8+ T-CELLS AFTER MURINE ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION

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Background: Acute graft-versus-host disease (aGVHD) is a severe and often life-threatening inflammatory complication of allogeneic hematopoietic cell transplantation (allo-HCT). aGVHD is mediated by alloreactive donor T cells attacking the gastrointestinal tract, liver, and skin of the host. Efficient strategies to improve aGVHD-related morbidity and mortality will rely on more precise methods than preemptive immunosuppression to consistently predict aGVHD and abrogate disease manifestation without exposing patients to an unwarranted risk for infectious complications. Recent insights into the multistep-pathophysiology of aGVHD provide a good basis for the development of new tests to identify individual patients at risk before the onset of aGVHD.

Aims: As pathologic T cell responses rely on spatiotemporally defined programs of T cell activation, acquisition of effector functions, and homing to GVHD target tissues it appeared attractive to assess receptor expression profiles of peripheral blood T cells as potential predictive markers.

Methods: Therefore, we characterized the surface receptor expression profile of peripheral blood donor lymphocytes early after allo-HCT in two independent murine models across minor histocompatibility antigens (miHAg) with multicolor flow cytometry. C57Bl/6 (H-2b, Thy1.1+) or B10.D2 (H-2d, Thy1.1+) T cells plus bone marrow cells were transplanted in conditioned (8Gy) miHAg mismatched BALB/c (H-2b, Thy1.2+) and syngeneic C57Bl/6 (9Gy) or BALB/c (H-2d, Thy1.1+) recipients. To identify suitable predictive markers, we compared the expression pattern of allo-HCT recipients to syngeneic HCT recipients and untreated wild type controls.

Results: Comparing a panel of T cell surface receptors, we found the homing markers CD44, CD45, and CD54 were highly up-regulated on allo-HCT recipients 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 we propose that alloreactive CD8+ T cells can be identified in miHAg allo-HCT recipients upon their homing receptor expression pattern as soon as to ten days before the onset of aGVHD.

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Thalassemias

E1570

SOLUBLE FORM OF TRANSFERRIN RECEPTOR IS ASSOCIATED WITH AGE AT DIAGNOSIS AND RISK OF THERAPEUTICAL INTERVENTION AND IRON OVERLOAD IN PATIENTS WITHOUT TRANSFUSION-DEPENDENT THALASSAEMIA

Aims: To investigate the soluble transferrin receptor and ferritin levels in a cohort of patients with β-thalassemia major (β-TM) in order to measure iron levels in the different organs.

Methods: 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.

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 complacent 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, 64% specificity, 70% positive predictive value and 88% negative predictive value.

Background: The soluble transferrin receptor (sTfR), that fully reflects the marrow erythropoietic activity, was found to have not only a striking diagnostic accuracy in predicting the risk of extramedullary haematopoiesis (EMH), but also in scoring disease severity in non-transfusion-dependent thalassemias (NTDT).

Aims: We retrospectively evaluated the relationship between sTfR and some fundamental events in the life and in the management of patients with NTDT.

Methods: We considered 111 NTDT patients with four genetic entities of NTDT: homozygous or compound heterozygous state for β-thalassemia, triplicated a mutation associated with β heterozygosity, deleitional HbH, and combination of a β defect plus a β chain variant. sTfR was measured with a commercially available kit. A group of patients was enrolled in the Myocardial Iron Overload in Thalassaemia (MIOT) network and underwent hepatic iron overload assessment by the T2 Magnetic resonance Imaging (MRI) technique.

Results: The group with homozygous or compound heterozygous for β-thalassemia had the higher sTfR levels. sTfR values were negatively related to age at diagnosis (R=-0.462, P<0.0001), and to age at first transfusion (R=-0.703, P<0.0001). At ROC curve a sTfR>5.3mg/L discriminated the patients with a previous history of occasional transfusions. sTfR values were significantly higher in splenectomized patients. sTfR values were negatively related to age at splenectomy (R=-0.328, P=0.044) and in unsplenectomized patients a significant positive correlation was found between sTfR values and spleen diameter (R=0.572, P=0.0001). sTfR values were negatively related to age at starting chelation therapy (R=-0.564, P=0.044). Patients never chelated showed significantly lower sTfR values than patients under chelation therapy (see Figure). sTfR values were significantly correlated with serum ferritin levels (R=0.321, P<0.0001), but not with LIC values.

E1571

LOW SERUM FERRITIN LEVELS DO NOT PROTECT FROM CARDIAC AND HEPATIC IRON IN PATIENTS WITH THALASSAEMIA MAJOR

Aims: The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in Beta Thalassaemia Major (TM).

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: sTfR levels 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 not 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.

E1572

ISCHEMIA MODIFIED ALBUMIN AS A MARKER OF OXIDATIVE STRESS IN CHILDREN AND ADOLESCENTS WITH Β-THALASSEMIA: RELATION TO LIPID PEROXIDATION, IRON OVERLOAD AND VASCULAR DYSFUNCTION

Aims: To measure the levels of IMA in 45 children and adolescents with β-TM compared with 30 healthy controls and assess its relation to lipid peroxidation, vascular complications and subclinical atherosclerosis.

Methods: β-TM patients without symptoms of heart disease were studied focusing on transfusion history, chelation therapy, serum ferritin, malondialdehyde (MDA) and IMA levels. Echocardiography was performed and carotid intimamedia thickness (CIMT) was assessed.

Results: IMA and MDA levels were significantly higher in β-TM patients compared with controls (p<0.001). IMA was higher among patients with heart disease and pulmonary hypertension (PH) risk than those without. Serum IMA and MDA levels were elevated among patients with serum ferritin ≥2500 µg/L compared with patients below this cutoff. TM patients complacent 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, 70% specificity, 88% positive predictive value and 99% negative predictive value.

Background: Patients with β-thalassaemia 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.
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 encountered in Greece. The frequency of thalassemia in our country is 1/60 and 1/40 of the population are carriers of the Hb S mutation. The rate of β-thal carriers could be as high as 15-20% in some areas. The risk of giving birth to an affected child depends on the incidence of the thalassemic gene and this may vary from 1/24 to 1/150 in married couples. The National Program for prevention of Thalassemia was established in 1973. Through population screening and prenatal diagnosis programs Greeks and immigrants are screened and counseled.

Aims: We report our findings on prenatal diagnosis of thalassemias and hemoglobinopathies in Northern Greece over a 15 year period (2001-2015).

Methods: During the 15 year period, a total of 33.837 subjects were screened individually or as couples at our Thalassemia Prevention Unit. There were 3.659 pregnancies at risk for thalassemia major offsprings and 84 for sickle cell disease ones. Prenatal diagnosis was started at 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).

Results: The remaining 42 pregnancies involved couples who were double heterozygotes for mutations that did not cause severe clinical disease and were exempted from prenatal diagnosis. The gene interactions were as follows β-thal/α thal, β-thal in combination with Hb Ε-Saskatoon or D- Punjab, HbE/HbE, Hb E-Saskatoon/or carrier of HbS, and Hb O/ Hb O, β-thal or α-thal in combination with D Punjab, Hb Brugg/β-thal, silent β-thal silent β-thal. 91% of the couples were of Greek origin, and 9% were immigrants from countries like Eritrea, Nigeria, Pakistan, Romania, VietNam and Thailand. We had an average of 15-32 prenatal diagnosis per year.

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.

E1574
SERUM N-TERMINAL PRO-BRAIN NATRIURETIC PEPTIDE LEVEL AND ECHOCARDIOGRAPHIC TISSUE DOPPLER ABNOMALITIES 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 anaemia 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 measurement of left ventricle (LV_TEI index) by TDI showed significant difference in cardiac function in cardiac iron overloaded patients reflecting early diastolic dysfunction. Myocardial performance index of the left ventricle (MIOT) network.

Aims: 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 THALASSAEMIA 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 liver iron overload.

Aims: We analyzed the impact of the presence of fatty liver in the parameters of iron overload among our patients with Non Transfusion dependent Thalassemia (NTTD).

Methods: 111 patients with NTDT were cross-sectionally evaluated; the diagnosis of liver steatosis was ultrasonography-based. In all patients serum ferritin levels and serum alanine aminotransferase (ALT) were 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 (MIOT) Network.

Results: Liver steatosis was frequently (35.5%) encountered among our patients with NTDT and was significantly more prevalent in males with respect to females (49.0% vs 24.6%, p=0.008). Patients with liver steatosis had significantly higher values of ALT, AST, ALT/AST ratio and ferritins than those without, but LCI values were comparable (Table 1). At ROC curve analysis, a LALT/AST ratio >0.89 predicted the presence of liver steatosis with a sensitivity=0.872 and a specificity =0.901 (P<0.0001). Overall, ferritin levels positively correlated with 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).

Table 1.

Summary/Conclusions: Our data show that liver steatosis affected also patients with NTDT and should be suspected in presence of a ALT/AST ratio >0.89. Recently, serum ferritin thresholds to predict clinically relevant liver iron concentrations for guiding chelation therapy when MRI is unavailable in patients with (NTDT) have been provided. Our data show that the presence of liver steatosis may lead to overestimate the magnitude of iron burden and may be responsible for anticipating or exceeding chelation treatment in patients with NTDT in absence of a LIC evaluation.

E1576
CIRCULATING CELL-FREE DNA (cfDNA) AND INEFFECTIVE ERYTHROPOIESIS IN BETA-THALASSEMIA INTERMEDIA

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Background: Low concentrations of circulating cell-free DNA (cfDNA) are found in the plasma of healthy individuals and increase in a number of conditions, 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 thalassemias, 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-thalassaemia, 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 plasma. cfDNA concentration determined fluorometrically using the fluorescent dye PicoGreen. Biochemical and hematologic parameters were determined in all patients as a part of laboratory routine. Reticulocytes and peripheral erythroblasts (EBL) were counted by automated procedures. Soluble transferrin receptor (sTfR) and growth differentiation factor 15 (GDF15) were also measured by ELISA assays. The aim of our study was to prospectively assess whether the Piga’s criterion had a prognostic role for adverse cardiovascular outcomes in TI patients.

Methods: We studied prospectively 168 TI patients (81 males, mean age 38.32 ±11.61 years) consecutively enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network. Standard cine steady-state free precession sequences were acquired and used for the calculation of biventricular function parameters (short-axis) and for the calculation of the thickness of the non-compacted and the compacted myocardium (three diastolic long-axis views) in all 16 segments. The maximal NC/C ratio was considered. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: Eight patients were excluded because a cardiac complication was present at the first CMR. The baseline mean age of the considered 161 TI patients was 38.32±11.61 years and 81 patients were males. The study population was divided into two groups: patients with Piga’s positive criterion (n=15, 9.31%) and with Piga’s negative criterion (n=146, 90.68%). No significant differences were found between the two groups in terms of demographic features and CMR parameters. The mean follow-up time was 57.50±21.87 months. Sixteen new cardiac events were recorded: 1 heart failure, 10 supraventricular arrhythmias and 5 pulmonary hypertension. Due to numerical reasons, it was possible to perform a Cox regression analysis only for arrhythmias and cardiac complications globally considered. Patients with Piga’s positive criterion had a significant higher risk of developing arrhythmias (hazard ratio=HR=7.19, 95% CI=2.02-25.51; P=0.002) and cardiac complications (HR=3.36, 95% CI=1.18-11.36, 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 availabl-
ability. Deficiency of both biochemical mediators promotes vasocstriction of the pulmonary vasculature resulting in further endothelial dysfunction, with subsequent intensified reduction of nitric oxide. The role of nitric oxide dysregulation is well-studied in non-transfusion dependent thalassemias and in sickle cell disease, but yet not very well-characterized in beta thalassemia major.

**Aims:** The aim of our work is to study the relation between intrinsic nitric oxide level and the evolution of pulmonary hypertension in beta thalassemia major.

**Methods:** This is a case-control study, including all patients with beta thalassemia major above 12 years of age, undergoing follow up in pediatric hematology 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 (TRJV >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 pathophysiologic effects of nitric oxide deficiency in beta thalassemia major e.g. its relation to coagulopathy and platelet aggregation are recommended.

E1579

Abstract withdrawn.

E1580

**SPECKLE-TRACKING ECHOCARDIOGRAPHY FOR DIAGNOSIS OF EARLY MYOCARDIAL DISEASE IN EGYPTIAN BETA THALASSEMIA MAJOR PATIENTS**

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**Background:** The new parameters of cardiac function, derived from two-dimen sional speckle-tracking echocardiography could be useful for an early diagnosis of cardiac involvement in transfusion dependent β-TM patients.

**Aims:** In this cross sectional study, our goal was to detect early myocardial disease in transfusion dependent β-TM patients using Echocardiography (Speckle Tracking) to assess its specificity and sensitivity in comparison with cardiac MRI T2*.

**Methods:** This cross sectional study included 30 transfusion dependent 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 2 years) Radiological investigation included Echocardiography (Tissue Doppler and Speckle Tracking); MRI T2* were done. Cardiac affection by speckled was defined as decreased longitudinal strain less than 11 percentage or affection of any segment less than 11 percentage.

**Results:** Cardiac affection by speckled echocardiography was found in 10 patients (33.3%), 8 of them (80%) had normal ejection fraction and normal shortening fraction, while 9 had iron overload by Cardiac MRI T2*. Patients with mean serum ferritin 2500 ng/ml in the last 2 years prior evaluation showed a significantly lower longitudinal strain (GLPSLAX) (P=0.043) which was further proved by a significantly negative correlation with the mean serum ferritin (P=0.002). No significant differences were found between both spelenomorized and non spele nomerized patients as regard speckle tracking echocardiographic measures. The ROC curve analysis revealed that GLPSLAX & GLPSLAXC and higher Ao Diam than those without cardiac iron overload (P=0.016, P=0.008, P=0.047 respectively). No significant difference between beta thalassemia patients with cardiac affection and those without cardiac affection as regard the duration of the disease, type and compliance of chelation therapy.

**Summary/Conclusions:** Although, Magnetic Resonance Imaging T2* technique is still considered the reference standard in myocardial iron overload, its diagnostic accuracy and a better achievement of an optimal glycemic control. Continuous glucose monitoring system (CGMS) enables more well defined. Continuous glucose monitoring system (CGMS) enables more physiologic effects of nitric oxide deficiency in beta thalassemia major. This effect could be related to the degree of hemolysis, iron overload and the duration of disease. Further studies on the adverse pathophysiologic effects of nitric oxide deficiency in beta thalassemia major e.g. its relation to coagulopathy and platelet aggregation are recommended.
mellitus. The percentage of diabetic patients diagnosed by CGMS was significantly higher than that with OGTT (p = 0.012). According to OGTT 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 patients with RBG ≥140mg/dL were noncompliant and 75% of patients on desferrioxamine therapy had RBGe140mg/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 regards GDF-15 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: In the diagnosis of early glycermic abnormalities (prediabetes) among patients with β-TM appears to be promising and superior to other known diagnostic modalities namely OGTT and HbA1c.

E1583

LEFT VENTRICULAR REGIONAL FUNCTION IN CHILDREN WITH BETΑ THALASSEMIA WITH NO CARDlAC MANIFESTATIONS (FOUR-DIMENSIONAL ECHOCARDIOGRAPHIC STUDY)

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Background: Early detection of myocardial dysfunction is essential for the management of patients with thalassemia. Four-dimensional echocardiography imaging technique that analyzes the motion of tissues in the heart may be useful for detecting subclinical cardiovascular disease.

Aims: To evaluate the 4-dimensional echocardiographic strain in children with beta thalassemia major and correlate it with other echocardiographic parameters.

Methods: This is a cross sectional cohort Study included 200 children, 1-18 years old. They were divided into: One hundred children with β-Thalassemia major with no clinical cardiac manifestations and 100 healthy children as a control group. They were subjected to the following investigations: Complete blood count, serum ferritin and Four-dimensional echocardiographic strains (Longitudinal, Circumferential, Radial and Area strains).

Results: There was no significant difference between the two groups as regard mitral annulus systolic velocity (S wave), E/A ratio and iso-volumic acceleration but the β group was significant difference as regard to ejection fraction, left ventricle mass, sphericity index and myocardial performance index. The mean values of Left ventricular Strains (Longitudinal, Circumferential, Radial and Area strains) were significantly lower in patients with thalassemia (-14.86±12.131, -8.01±3.829, 33.13±10.613, -19.45±6.866) than controls (-19.13±5.502, -16.72±3.933, 37.23±10.794) but there was significant difference with respect to positive correlation with 2-Dimensional strain.

Summary/Conclusions: Strain parameters of the left ventricle obtained by four-dimensional echocardiography can be a novel and promising technique for early detection of left ventricular dysfunction in children with thalassemia.

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: 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, 153±105,78 ng/mL. Mean GDF-15 level was 9672.57±7931,36 pg/mL. Mean duration of T2 MRI was 32.50±11.33 ms for heart and 4.87±3.78 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, 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.

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.

E1585

ASSOCIATION OF SP1 POLYMORPHISM IN THE COLLAGEN TYPE I ALPHA-1 (COL1A1) GENE WITH OSTEOPOROSIS IN CHILDREN WITH BETΑ-THALASSEMIA

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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 children with beta-thalassemia.

Aims: To study the relationship between SP1 polymorphism in the collagen type 1 alpha 1 gene and the development of osteoporosis in patients with beta thalassemia.

Methods: A prospective case control study was carried out in the Outpatient Clinic of Hematology Unit of Pediatric Department and Clinical Pathology Department at Zagazig University Hospitals on forty thalassemic patients (21 females &19 males) aged 6-18 years during their regular follow-up visits (22 patients with thalassemia major and 18 with thalassemia intermedia) and forty age- and sex-matched healthy children as a control group. All patients and control were subjected to full medical history, thorough clinical examination and laboratory investigations in the form of complete blood count, Hep electrophoresis, Calcium level Serum , alkaline 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 genotye there was high percentage of heterozygous Ss (G/T) and homozgyous ss (T/T) genotypes 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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Background: Hemoglobin (Hb) is a protein responsible for oxygen transport from lungs to the entire body. It is composed by four globular subunits - the globins - each with a central core containing a heme molecule. Globins are encoded by the α- and β-globin gene clusters located at 16p13.3 and 11p15.5, respectively. The pattern of globin gene expression during development is precisely controlled by the interaction of cis-regulatory genomic regions (located in close proximity to and far from genes) with trans-activating/silencing factors within permissive chromatin domains. Distally upstream of the α-globin genes there are four multispecies conserved sequences (MCS-R1 to R4) which are critical to maintain the downstream globin cluster. Deletions removing the α-globin genes and/or their distal MCSs give rise to α-thalassemia, one of the most common genetic recessive disorders worldwide, due to a reduced rate of α-globin chain synthesis. The severity of the pathology is variable ranging from a very mild microcytic hypochromic anemia to a moderately severe anemia associated with the formation of αβ4 tetrads resulting in HbH disease or an even higher reduction or complete absence of α-chains resulting in hemoglobin Bart’s hydrops fetalis, a condition generally incompatible with life.

Aims: The main objectives of this work were to characterize the molecular lesions underlying ten Portuguese cases of unusual α-thalassemia/HbH disease and to establish the origin and functional consequences.

Methods: After exclusion the most frequent molecular lesions associated with α-thalassemia, Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA MLPA P140B HBA kit (MCR-Holland) was used to search for DNA deletions in the subtelomeric region of chromosome 16p. Additionally, specifically designed synthetic MLPA probes, as well as gap-PCR and Sanger sequencing were performed for more accurate deletion breakpoint mapping.

Results: We have found five distinct deletions and one indel, all in heterozygosity. The deletions range from approximately 3.3 to 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 who revealed to be a very unusual case of acquired alpha-thalassemia as possible originating from a complex rearrangement involving chromosome 3.

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 deletion carriers to provide patients with appropriate genetic counseling.

E1586

Abstract withdrawn.

E1588

VALUE OF HBA2 IN THE DIAGNOSIS OF BETA-THALASSEMINA MINOR “ATTENTION TO THE GRAY ZONE”

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Background: The homozygosity for the alternative splicing mutation HBB: IVSI-6 (C>T) is the most frequent genotype of beta thalassemia intermediate in our population and was even termed “beta thalassemia intermediate type Portuguese” (Tamagnini et al., 1983). The IVSI-6 (C>T) carriers (heterozygous) are characterized by mild hypochromia and microcytosis, with a moderately increased in HbA2, that may be even less than 3.5%. The correct identification of these carriers is important, especially when facing a couple who intends to have children.

Aims: To evaluate the percentage of individuals with hypochromia and microcytosis and Hb A2 between 3.2% and 3.4%, which are beta thalassemia carriers, alerting for the need to adapt the cut-offs of HbA2 values to the genetic background of different populations.

Methods: Methodological search of all the consecutive individuals evaluated in our laboratory from January 2007 to January 2016. The inclusion criteria were the simultaneous presence of hypochromia and microcytosis (adjusted to the age) and HbA2 values between 3.2% and 3.4% inclusive. The exclusion criteria were the presence and/or clinical information of sideropenia or sideropenic anemia, hemoglobin variants or alpha thalassemia. Sequencing of the entire HBB gene was preformed by Sanger Sequencing.

Results: Respecting the inclusion and exclusion criteria we have identified 43 individuals with hypochromic and microcytic anemia, HbA2 ≥3.2% and ≤3.4%, in which the HBB gene mutations were screened. Among the 43 subjects, nineteen preent HbbA2=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 were classified as probable alpha thalassemia and suggested continuation of the study, if warranted.

Summary/Conclusions: We have identified 14/43 (32%) individuals as beta thalassemia carriers who, for the conventional cut-off of HbA2 ≥3.5%, would not have been diagnosed. Based on this data, we propose that individuals with hypochromia and microcytosis, with normal or slightly elevated RDW, without sideropenia, with HbA2 between 3.2-3.4%, should be screened for mutations in the HBB gene, in order to rule out beta thalassemia carriers due to Beta+ mutations. As HBB IVSI-6 (T>C) mutation is one of the most frequent beta thalassemias mutations in Portugal, and in Mediterranean basin, it is necessary to screen for it. The classic rule of HbA2=3.5% for the diagnosis of beta thalassemia minor may underdiagnose this pathology and lead to an incorrect genetic counseling.

E1589

DIAGNOSIS OF HEMOGLOBINOPATHIES BY CAPILLARY ZONE ELECTROPHORESIS: EXPERIENCE WITH 925 CASES

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Background: Hemoglobin capillary zone electrophoresis is a relatively newer technique as compared to HPLC for detection of abnormal hemoglobin. We share our first hand experience of using Capillary 2 Flex piercing instrument for diagnosis of hemoglobinopathies as a primary diagnostic modality.

Aims: The main aim was to evaluate a new technology for diagnosis of hemoglobinopathy.

Methods: The capillary 2 Flex piercing instrument with Phoresis software for hemoglobin electrophoresis for capillary electrophoresis was evaluated during our study period of 1 year. A total of 925 sample runs were included in the analysis. The equipment was assessed on the following parameters: ease of operation, pre-analytical factors, identification, quantification and precision of hemoglobin variants including the rare variants. Further, we evaluated if capillary zone electrophoresis can be used as a simple method for diagnosis of hemoglobinopathies.

Results: The automation provided by capillary zone electrophoresis eased the problem of errors during sample preparation. The option for low sample volume mode is a great help in samples from children. The instrument could readily identify all common hemoglobins. The diagnosis was straightforward in 829 (89.7%) cases. In the rest 96 (10.3%) cases, the sample was required to be rerun because it lacked Hb A or Hb A2. This posed inconvenience because the electrophoretic zones get displaced and have to be derived after mixing it with normal sample. The machine is not specifically standardized for cord blood samples hence we did not perform testing on neonatal cord blood sample. The instrument could separate and identify Hb E from Hb H with very good precision and a good scorer over HPLC, however, we found mild high Hb A2 both in heterozygous and homozygous Hb E cases (heterozygous Hb E, n=28 mean Hb A2=3.9% and homozygous Hb E, n=7 and mean Hb A2=4.2%) leaving the doubt whether some adducts are still left. Identification of small peaks of Hb H could be difficult and requires other modalities to confirm. Two cases where Hb H was strongly suspected clinically and Hb H inclusion test was positive showed small peaks of Hb H (1.2% and 0.9%) on HPLC. Hemoglobins falling into the same zone (eg Hb D- Punjab and Hb Q India) needed identification with second modality. Whenever encountered with problem of identifying certain abnormal peak, we resorted to HPLC for confirmation. Spectrum of hemoglobin variants encountered (n=298 cases, rest 627 showed normal results) in the study is listed in table below.

Table 1.

<table>
<thead>
<tr>
<th>Hb Variants</th>
<th>No of cases (n=298)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Beta thalassemia</td>
<td>35</td>
</tr>
<tr>
<td>2. Hb E</td>
<td>38</td>
</tr>
<tr>
<td>3. Hb E-0</td>
<td>5</td>
</tr>
<tr>
<td>4. Hb E-2</td>
<td>4</td>
</tr>
<tr>
<td>5. Hb E-4</td>
<td>1</td>
</tr>
<tr>
<td>6. Hb H</td>
<td>7</td>
</tr>
<tr>
<td>7. Hb H-0</td>
<td>6</td>
</tr>
<tr>
<td>8. Hb H-2</td>
<td>3</td>
</tr>
<tr>
<td>9. Hb H-4</td>
<td>1</td>
</tr>
<tr>
<td>10. Composed heterozygotes (eg Hb E-beta thalassemia or Hb E-beta thalassemia)</td>
<td></td>
</tr>
</tbody>
</table>

Summary/Conclusions: Capillary zone electrophoresis is an alternative method for hemoglobinopathy. However, since the diagnosis of Hemoglobinopathies mandates confirmation by a second method, HPLC cannot be replaced completely. Based upon the availability, workload and cost effectiveness, any of these two methods can be used as primary modality.
Thrombosis and vascular biology

E1590
RELEVANT ROLE OF VON WILLEBRAND FACTOR-ADAMTS13 AXIS IN HEPATIC ISCHEMIA-REPERFUSION INJURY
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Background: Hepatic ischemia-reperfusion (IR) 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 IR 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 IR injury. To test this hypothesis, we have used a mouse experimental model of hepatic IR injury.

Methods: Mice were anesthetized with sodium pentobarbital and a midline laparotomy was then performed on a heating pad. Blood supply for left lateral and median lobes of liver (approximately 70% of the liver mass) was interrupted by crossing to the hepatic artery and portal vein with a microvascular atrumatic clip for 90 min. Then a clip was taken off to provide 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. Histological analysis and histofluorescence of liver tissue were performed.

Results: As compared to WT mice, restoration of hepatic blood flow was significantly greater in VWF-KO mice at 24 h after reperfusion (WT: 61 ± 17 vs KO: 87 ± 17%, expressed as the percentage of pre-ischemic value). Consistent with the hepatic blood flow, the time-course analysis of serum alanine aminotransferase (ALT) at several time points after reperfusion revealed the lesser liver damages of KO mice (WT: 689±3270 and 131±626 IU/L vs KO: 3043±1320 and 478±330 IU/L, at 3 h and 24 h after reperfusion, respectively).

In addition, histological analysis confirmed that neutrophil infiltration in the liver tissue of KO mice was significantly reduced as compared to WT mice at 24 h after reperfusion. These impaired hepatic blood flow and ALT values as well as intensified neutrophil infiltration in WT mice were significantly improved to an extent comparable to those of KO mice by the bolus injection of recombinant human ADAMTS13 (3 µg/mouse equivalent to 2800 U/kg, n=12) just prior to the IR operation.

Summary/Conclusions: Our results altogether indicate that VWF-dependent inflammatory responses with neutrophil recruitment at ischemic sites are involved in pathophysiology of hepatic IR injury, and functional regulation of VWF by ADAMTS13 may serve as a promising therapeutic option for hepatic IR injury.

E1591
THE IMPORTANCE OF THE FULL BLOOD COUNT, JAK II AND ADAMTS13 TESTING IN STROKE EVALUATION: A REVIEW OF 619 CONSECUTIVE PATIENTS WITH THROMBOCYTOPENIA. A 6 YEARS SINGLE EXPERIENCE CENTER
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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 thrombosis in a younger cohort.

Aims: To analyze the incidence of PRT, describe the clinical characteristics and management of these patients and identify the risk factors of PRT.

Methods: We retrospectively reviewed peripherally inserted central catheters (PICCs) related thrombosis in 230 patients with hematological malignancies. A 6 YEARS SINGLE EXPERIENCE CENTER

Results: The median age was 58 years (14-86) and 55.7% of the patients enrolled in the study were male. The most frequent hematological malignancies were: Non-Hodgkin’s lymphoma (NHL=105; 45.7%) myeloid malignancies (acute myeloid leukemia and myelodysplastic syndrome=100; 26.1%), acute lymphoblastic leukemia (ALL=22; 9.6%), multiple myeloma (MM=19; 8.3%) and Hodgkin lymphoma (HL=17; 7.4%). In 188 patients (82%), PICC was tunneled when the active disease was presented. Only 51 patients (22%) received thromboprophylaxis based on low molecular weight heparin (LMWH=27), aspirin (ASA=21) or vitamin K antagonist (VKA=3). PICCs were tunneled guided by US in 127 patients (55.2%), and the main location of tip catheter was in cava-right atrium region (66%). The overall incidence of PRT was 7% (n=16). The main diagnoses related to PRT were ALL (6), NHL (5), HL (5), and MM (6). All dissections were symptomatic. All except three patients with tunneled PICCs (15/16=94%) suffered a PRT. Fourteen patients (88%) were treated by chemotherapy based in L-asparaginase (L-ASA), immunomodulatory drugs or other treatment combined with corticosteroids. The median onset of PRT was 26 days, (range: 0-230) and 8 of them (50%) in the first 30 days after insertion. In 11 cases (69%) thromboprophylaxis was removed within 72 hours of PRT and treated with LMWH to a median of 4 months (range: 1-11). During follow-up, no patient had progression of thrombosis, or pulmonary thromboembolism. Finally, in the univariate analysis ALL, HL and L-ASA had significant impact on
E1593
A STUDY OF VENOUS THROMBOEMBOLISM SUSCEPTIBILITY LOCI FACTOR XI, ABO AND FIBRINogen IN A PORTUGUESE POPULATION SAMPLE
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Background: Venous thromboembolism (VTE) is a multifactorial disease caused by genetic and environmental factors acting on an individual's risk factors and complex gene-environment interactions. VTE results from the development of a thrombus, usually in the deep veins of the leg (deep vein thrombosis, DVT) that can subsequently embolise to the lung (pulmonary embolism, PE). Classical inherited risk factors for VTE in European-ancestry populations include protein C and S deficiencies, factor V Leiden and prothrombin gene mutation (FII G20210A). Several other common and low-frequency susceptibility variants, mainly single nucleotide polymorphisms (SNPs) in loci ABO, FXI, FII, FVG, GP6, KNG1, PROCR, SLCA4A2, STXBP1, TSPAN15 and VWF, have been also found robustly associated with VTE. However, in the Portuguese population the genetic background for VTE for most of these genetic susceptibility variants remains to be evaluated.
Aims: To investigate the association of five SNPs in the loci ABO (rs2519093 and rs8176719), FXI (rs2036914 and rs2289252) and FGG (rs2066865) with VTE in a sample of Portuguese patients.
Methods: A retrospective (2012-2015) case-control study with 119 cases of unprovoked VTE and 148 healthy controls of Portuguese origin was conducted, to evaluate allele frequencies of the five VTE risk alleles in the Portuguese population and to assess the association between these alleles and the risk for VTE. FXI (rs2036914 and rs2289252) and FGG (rs2066865) SNPs were genotyped by real-time PCR with TaqMan probes. ABO rs2519093 and rs8176719 SNPs were genotyped by restriction fragment length polymorphism (RFLP). PLINK software was used to determine the allelic frequencies, concordance with Hardy-Weinberg equilibrium and association between risk alleles and VTE through logistic regression, in the additive model, estimating OR with 95% confidence intervals (95% CI) and p-values. The association between the cumulative number of risk alleles and the risk of VTE was assessed using Pearson χ² using the Simple Interactive Statistical Analysis software (SISA).
Results: The estimated risk allele frequencies in the overall study population sample were: 0.212 for FGG 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 genotype distributions were in agreement with the Hardy-Weinberg equilibrium (HWE) for all SNPs. The risk allele frequencies of all SNPs did not deviate significantly from the HWE, with the exception of ABO rs2519093, which had a lower frequency in the overall study population.
Summary/Conclusions: Our data suggest that the alleles FGG rs2066865 T and ABO rs8176719 C may contribute to the VTE susceptibility in the Portuguese population. The absence of significant associations for the remaining loci could be the result of a limited statistical power, consequence of a modest effect size of polymorphisms or lower sample sizes, or because of differences in genetic backgrounds between populations.
E1594
PEDEtiATriC VeNOUS tHROMBOEmBOLiSM: iNCiDenCe, riSk FACTORS AND MANAGeMENT OF HOSPITALIZED PATIENTS IN A tERtiARY CARE tEACHING HOSPITAL
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Background: Venous thromboembolism (VTE) is a considered rare event in childhood. In spite of this, the incidence of VTE is on the rise in hospitalized patients. Medical progress in the treatment of critically ill patients has increased the use of central venous catheters (CVC) and interventional procedures, especially in children with cardiac defects and malignant disease. Therefore VTE is increasingly recognized as a major secondary complication of advanced tertiary care in infants and children.
Aims: To study the incidence, demographics, risk factors, diagnostic tests, therapy, and complications of pediatric acute VTE in our tertiary care hospital.
Methods: A retrospective single-center study of patients<18 years of age who were discharged from January 2014 to December 2016 by using diagnostic codes for acute VTE from our hospital database. We studied demographic characteristics, clinical presentation, diagnostic tests, risk factors, treatment strategies and outcome.
Results: We report an incidence rate of 10.7 cases per 10,000 patient-years (70 acute VTE events / 21,892 discharge cases over a 3-year period). Patients were predominantly male (57%). Mean patient age was 3.3 years, with the greatest proportion of cases (35.5%) in the infants (<1 year old), 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 (NCC) diagnoses were more frequent intracranial in 35.3% of the cases. In those with an 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 triggers such as infection (12 cases) and use of aspiraginase (2 cases) were also reported. Most patients were not tested for thrombophilia (n=44, 62.9%) since they were classified as provoked VTE and from those who were tested 10% were diagnosed with a thrombophilia. AML and AML-related one patient initiated anticoagulant therapy: 78.6% (n=52) were initially treated with low molecular weight heparin (LMWH) and while most continued treatment with LMWH, 8.6% (n=6) received vitamin K antagonists and 8.6% received direct oral anticoagulants. LMWH dosing was adjusted using anti-Xa assays (AXA) in 85.7% of cases, documenting a median of 5 AXA per patient, out of which 3 were within therapeutic range. Mean duration of treatment was 5.8 months. Recurrence rate was 17%, half of which were in patients with CVC-VTE. On the other hand, bleeding rate was 15.7% most of which were mild (10%) or provoked bleeds (4.3%). Mortality was 10%, although cause of death was not directly related to VTE in any of the cases.
Summary/Conclusions: The incidence of VTE in children is a substantial complication arising from tertiary care hospitalization where critically ill infants are at greater risk. Potential risk factors of VTE include use of CVCs, patients with complex congenital heart defects, surgical procedures, infection and malignancy. Further studies on VTE prophylaxis and identification of VTE predictors in a critical care setting are required.
E1595
CELL-BASED EVALUATION OF CHANGES IN COAGULATION ACTIVITY INDUCED BY ANTIINFLAMMATORY DRUGS ACTIVITY FOR THE TREATMENT OF ACUTE MYELOID LEUKEMIA
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Background: Indirubin (IDR) is a natural compounds (Arac) and luteolin (Amb0) are drugs for treatment of acute myeloid leukemia (AML). In leukemic cells, the incidence of venous thromboembolism or disseminated intravascular coagulation is associated with induction chemotherapy.
Aims: How some drugs for the treatment of AML affect the procoagulant activity is unclear. Thereby, in this study, we investigated the procoagulant effects of IDR in comparison with Arac and Amb0.
Methods: Procoagulant effects of IDR, Arac, and Amb0 were investigated in a vascular endothelial cell line Eha926 and AML cell lines HL60 (AML M2), NB4 (AML M3, APL), and U937 (AML M5), focusing on tissue factor (TF), phosphatase and tensin homolog (PT), and thrombomodulin (TM). Normal human plasma-based recalcification time assay, flow cytometric analyses, and RT-PCR are applied for the evaluation.
Results: IDR induced procoagulant activity on the surface of vascular endothelial and AML cell lines. Expression of TF antigen, TM antigen, and PS were induced by IDR on the surface of each cell line, whereas expression of TF and TM mRNAs were unchanged. Increased TF and PS expression may overcome increased TM expression and the overall effect may be procoagulant. Conversely, Am0 decreased TF expression and procoagulant activity, and increased TM expression on NB4 cells. In NB4 cells, we observed downregulation of TF mRNA and upregulation of TM mRNA by Am0. But Am0 did not sufficiently inhibit anticoagulant activity on NB4 cells when applied simultaneously with IDR.
Summary/Conclusions: These data suggest IDR may induce procoagulant activity in vessels by apoptosis through PS expression and/or TF expression on vascular endothelial and AML cell lines. Am0 may suppress procoagulant activity by increased TM expression and downregulation of TF expression and induction of TM expression. Our methods could be useful to investigate changes in procoagulant activity induced by antiinflammatory drugs.
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PRT (LLA/ OR 15,75; CI 2.91-85,12; p= 0.001; HLR: OR 9; CI 1.38-58,78; p=0.022; LASA: OR 7,82; CI 2.15-24,95; p=0.010). However, in the multivariate analysis, HL was the only risk factor of PRT (OR 8,38; CI 1.05-66,5; p=0,044).
Summary/Conclusions: Patients with HL may be predisposed to developing PRT compared with those with other types of hematological malignancies. Identifying the mechanism underlying this relationship will require further study.
Methods: identifying those patients with hospital-associated thrombosis (HAT), defined as patients having had a hospital inpatient episode, including day case surgery and admissions of 4-24 hours, in the 90 days prior to their VTE episode. 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 preventable standard patient >90k. Of those 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 outcome by current techniques. Key errors implicated are failure to perform a timely VTE risk assessment and action with appropriate thrombo-prophylaxis. Full integration of electronic patient records with electronic prescribing modules may reduce further these errors.

E1596
DESRIPTION OF THROMBOTIC EVENTS AND/OR PREGNANCY LOSS ES IN A COHORT OF HOMOZYGOUS CARRIERS FOR THE C46T POLYMORPHISM IN THE F12 GENE

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Background: The intrinsic pathway of coagulation is initiated by a serine protease named factor XII (FXII) in a reaction involving the contact system and triggers fibrin formation through activation of factor XI. In vitro, FXII triggers activation of the classic complement pathway and initiates the fibrinolytic system via plasma kallikrein mediated urokinase activation, whereas in vivo its role is still uncertain. F12 C46T polymorphism is located in the 5'-untranslated region of the F12 gene (F12 C46T) is associated with lower levels of FXII. Its frequency varies widely across populations and ethnic groups, ranging from 0.18 in the Spanish population to 0.67 among Japanese. Homozygosity for the C46T polymorphism of the F12 gene has proved to be an independent risk factor for thrombosis and unexplained recurrent spontaneous abortion. However, the precise role of this polymorphism as a thrombotic risk factor is controversial, and the evidence for an association between F12 C46T, venous thromboembolism (VTE) and myocardial infarction is weak.

Aims: To describe the occurrence of thrombotic events and/or pregnancy losses 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 assays using QIAGEN RPLAB Real-Time PCR System were used to determine 10% 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, 28.5% and 12.9% had one and more than one pregnancy loss respectively. Additional thrombotic risk factors were found in 60% of women with recurrent losses. One (43%) or more than one (46.7%) additional thrombotic risk factors were found in patients with any thrombotic event. Presence of one or more CVRF was 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: In 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 associated with pregnancy losses. Further studies are needed to clarify the real contribution of F12 C46T to thrombosis and pregnancy losses on prospectively selected patients.

E1597
ANALYSIS OF CHARACTERISTICS OF HOSPITAL ASSOCIATED THROMBOSIS

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Background: Hospital associated thrombosis (HAT) is now commonly monitored but expected targets of HATs remains poorly reported.

Aims: We analysed HATs in our hospital group over a 40 month period to assess any trends or patterns of HAT incidence and characterics over time.

Methods: Our hospital group includes 3 hospitals across our hospital group, identifying those patients with hospital-associated thrombosis (HAT), defined as patients having had a hospital inpatient episode, during case surgery and admissions of 4-24 hours, in the 90 days prior to their VTE episode. Root cause analysis was undertaken on these cases, recording information of the index episode and any associated thrombotic risk factors. Decisions to stop and restart anticoagulation were recorded. Of 2222 VTEs were identified (1051 PE's and 1178 DVTs) of which 581 (26%) HATs were identified (312 PE's, 269 DVTs). This represents an excess of PE's over the expected rate based on total VTE distribution between PE and DVT (p=0.0002 Fishers exact test). The majority of patients had a medical (non-surgical) index admission with 58.5% admitted as acute medical admissions and 41.5% surgical admission (trauma and orthopaedics 18.4%, general, vascular and GI surgery 12.2%, urology 4% and Gastroenterology and anaesthesiology 1%). Not all support these noso. In 526 HAT cases, root cause analysis (RCA) revealed that 101 (19.2%) were deemed preventable and 367 (69.8%) were not thought to be preventable. The remaining 57 cases had the index admission outside of our trusts, largely having been admitted via emergency procedures which were not recorded. Of 394 HAT cases with sufficient data, 80 (20.3%) had a preventable cause, 27 receiving insufficient TP, 9 receiving delayed TP, 26 having no TP given though indicated and 18 not having a VTE risk assessment. Some cases of insufficient TP were deemed preventable standard patient >90k. Of those 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 outcome by current techniques. Key errors implicated are failure to perform a timely VTE risk assessment and action with appropriate thrombo-prophylaxis. Full integration of electronic patient records with electronic prescribing modules may reduce further these errors.

E1598
THROMBOSIS DURING INFANCY AND NEWBORN PERIOD: AN UNRESOLVED ISSUE

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Background: Reported incidence of thrombosis is higher among newborn infants that can be explained by age related deficiency of anticoagulants, overproduction 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 22 thrombotic events during the infancy period involving 55 patients (10 infants<1-12 months and newborns<1 month) with thrombosis in our center.

Methods: Our database revealed 752 children having various types of thrombosis between January 2003 to December 2015 and 77 out of 752 were diagnosed as thrombosis under one year of age. We retrospectively evaluate their clinical and laboratory results for 55 thrombotic patients. Thrombotic risk factors included inherited and acquired hypercoagulable states, catheter, infection, trauma, surgical operations were also recorded.

Results: There were 51 male and 26 female with a median age of 4 months (0-12 months) in this group. Among 77 thrombotic events 22 (28%) were observed during the neonatal period (1 month) with a male predominancy (15, 68%) and from those 22 events 2 arterial thrombosis (purpura fulminans(1), cerebral(1)) whereas 4 intracardiac, 5 sinus venous and 11 venous thrombosis (deep veins(4), renal veins(3), portal veins(3) cerebral vein(1)) were noted. In 2(9%) cases thrombosis was diagnosed on the first day of life and 11 out of 22 patient had underlying risk conditions such as prematurity(3), perinatal hypoxia(2), necrotizing enterocolitis(1), congenital cardiac disorders(3),congenital nephrotic syndrome(1) and adrenal insufficiency(1).Moreover 6 out of these 22 thrombotic event catheter insertion was the associated risk factor and 4/22 had infection. Factor V Leiden mutation was found to be 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 treatment in 5 case with infrarenal iliac arterial thrombosis(2),femoral arterial(3),abdominal aortic thrombosis(1).During the follow up period 1 patient had an amputation, 5 patient deceased; one because of sepsis and the rest 4 had primary dissease and thrombosis. The site of location in 55 thrombotic events during the infancy period involved deep venous thrombosis (22), cerebral sinus venous 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.Initital treatment choice was LMWH in 25(45%) and during the follow up 10 had TPA, 10 had warfarin, 21 resolved, 10 had parsiel thrombolysis, 4 deceased and 10 loss to follow up.

Summary/Conclusions: During the first month of life thrombotic complications is 40 times higher than at any other pediatric age. As previously reported venous thrombosis which mainly affect the limbs, the right atrium and renal veins are more frequently seen than arterial thrombosis in newborn infants with a male 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 thrombotic treatment was tend to be used more commonly in the neonates without any complication.
Background: Soluble fibrin monomer complexes (SFMC) are the early marker of thrombophilia that represent the complexes of monomeric fibrin with fibrinogen or their products of degradation (FDP). SFMC levels are not directly affected by therapy with thrombolytic agents. Detection of SFMC formed due to the activation of blood clotting by thrombin reveals a pathological process in the early, preclinical stages.

Aims: We explored the quality difference between the SFMC fraction obtained from acute ischemic stroke patients and one year post acute phase of stroke in the absolutely the same patients.

Methods: SFMC fraction was obtained from each tested groups: 35 healthy donors as well as 66 patients with atherosclerotic ischemic stroke (AIS) and 56 patients with cardiometabolic 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% a-phenanthroline per 5 min. For Size-exclusion chromatography, SFMC in vol/ml was applied on Healthcare Life Sciences "HiLoad 16/60 Superdex 200 pg" column.

Results: Results suggest presence of proteins with Mr from 45 up to 330 kDa in SFMC fraction. The content of SFMC was similar for all stroke fractions with some exception. The difference between results of separation of stroke factions 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 chromogram of SFMC are common for all tested fractions and were verified only in their height. Accordingly, the most widely represented variations peaks for AIS, even a year after stroke soluble fibrin monomer complex content was higher comparing to the healthy donors index. Healthy donors also had some of these complexes, but in trace amounts. For acute CIS situation was similar as for AIS, but past one year it get closer to healthy donors.

Figure 1.

Summary/Conclusions: It was shown that development of ischemic stroke accompanied by the formation of SFMC in the bloodstream that could take part in disease complication.

E1600

EVALUATION OF A RAPID NANOPARTICLE-BASED LATERAL FLOW IMMUNOASSAY (STIC EXPERT HIT) FOR THE DIAGNOSIS OF HEPARIN-INDUCED THROMBOCYTOPENIA IN A CARDIOTHORACIC HOSPITAL

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Background: Heparin Induced Thrombocytopenia (HIT) is a severe complication of heparin anticoagulation treatment that could be life threatening. HIT diagnosis is therefore of crucial importance in clinical practice especially for the cardiothoracic patients that are often exposed to heparin before surgery (e.g. during a PTCI). Laboratory testing for the presence of IgA, IgM and IgG or IgG only antibodies against PF4/Heparin (namely HIT antibodies) along with the 4Ts scoring system (Thrombocytopenia, Time of platelet count fall, Thrombosis, Other cause of thrombocytopenia) is used to evaluate the probability of HIT syndrome. At the Onassis Cardiac Surgery Center the methods for routine laboratory testing for HIT comprise Enzyme-linked Immunoassay testing for IgG, IgA, IgM HIT 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 performed on plasma from 35 patients from July 2016 until January 2017 and the reading was done independently by two different technicians or biologists.

Gists. The diagnosis of HIT was confirmed when both H/PF4 ELISA and the 4Ts score were positive.

Results: In 22 cases the Stic Expert was negative for the presence of IgG H/PF4 antibodies in the patients’ plasma and HIT syndrome was easily excluded in combination with the 4Ts. In the rest 13 cases the rapid test provided 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 "LIA" were found not to have HIT syndrome in combination with the ‘4Ts’ scoring system.

Summary/Conclusions: In conclusion the Stic Expert HIT was useful for the quick exclusion of HIT (along with the 4Ts scoring system) when emergency HIT diagnosis is needed in 34% of the cases and then H/PF4 ELISA was performed in the rest 13/25 (66%) of the cases, laboratory testing for HIT was much more complicated and time consuming since ELISA or other assays (i.e. HIPA test) had to be performed. Nevertheless all 13 patients were found not to suffer from the HIT syndrome with the ‘4Ts’ scoring system.

E1601

AUDIT OF ‘DOOR TO NEEDLE’ TIME IN ADMINISTRATION OF PROTHROMBIN COMPLEX CONCENTRATE TO PATIENTS REQUIRING URGENT REVERSAL OF ANTICOAGULATION

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Background: Anticoagulants are used to treat or prevent thrombotic events but their most worrying side effect is major haemorrhage. The British Committee for Standards in Haematology (BCSH) recommend reversal of major/life threatening bleeding in both VKAs and DOACs with Prothrombin Complex Concentrate (PCC).

Aims: We aim to reduce delays in the administration of PCC in our trust and introduce the term ‘Door To Needle’ time (DTN) in the context of anticoagulant reversal.

Methods: We analysed the DTN in bleeding anticoagulated patients defined as time of 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 39%. The remaining indications (25%) were pre-urgent procedure/surgery, and soft tissue haematoma. The average time for recognition of haemorrhage was 3 hours 20 minutes (range 4 minutes to 21 hours 27 minutes), and the DTN was 4 hours 50 minutes (range 33 minutes to 13 hours 24 minutes), which means an estimated average of 6 hours 27 minutes (range 2 hours 49 minutes to 13 hours 59 minutes) between hospital admission and receiving PCC. Six of the total number of patients died within 30 days of hospital admission, 4 taking on Warfarin and 2 taking on DOACs.

Summary/Conclusions: This audit demonstrates the continuing delays between recognition of major/life-threatening bleeding events and receiving PCC since previous audits despite raising staff awareness. We plan to introduce the term DTN in the context of anticoagulant reversal, store PCC in the emergency department pharmacy cupboards (as a PoM) as opposed to blood bank, and introduce a reporting system ‘Serious Hazards of Warfarin (SHOW)’ which may further reduce delays, morbidity and mortality.

E1602

THE IMPORTANCE OF PLATELET MEMBRANE FLUIDITY AND OXIDATIVE STRESS IN THROMBOTIC COMPLICATIONS ACQUIRED BY CHRONIC MYELOPROLIFERATIVE NEOPLASMS PATIENTS

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Background: Patients with chronic myeloproliferative neoplasms (MPNs) and chronic myeloid leukemia (CML) have a variety of structural and functional abnormalities of platelets. Many of them have thrombotic or hemorrhage complications. Platelet function is influenced by changes in membrane fluidity (MF) which has an important role in the expression of platelet receptors, modulating the activity of protein membrane.

Aims: The importance of reactive oxidative species (ROS) in alteration function of platelet membrane and expression of platelet receptors in patients with MPNs and CML.

Methods: We present a retrospective study on 36 cases MPN (20 Jak2-positive MPN) and 24 CML admitted in Colentina Clinical Hospital Bucharest. The determination of platelet membrane fluidity was performed by fluorescence anisotropy measurements using as marker 1-(4-trimethylammoniumphenyl)-8-phenyl-1,3,5-hexatriene p-toluenesulfonate (TMA-DPH). We analyzed the fluorescence anisotropy of platelet membrane and correlate the result of with a
different kind of treatment. Production of ROS was examined using fluorospectroscopy method with DCFDA and Fluorolight spectrophotometer. Platelet receptor expression was analyzed by flowcytometry method studying adhesion marker (CD 42 and CD 43) and aggregation marker (CD 61, CD 41).

Results: Patients with MPN and JAK2 mutation present a high level of fluorescence anisotropy as compared to the JAK-2 negative group. Median value for JAK2 positive group 147.2 ± 2.95% CI for median value 151.75-150.6) vs JAK2 negative MPN group 130.8 ± 2.12% CI for median value 138.65-140.6). 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 with MPN and CML patients compared with healthy controls. CML patients in accelerate or blastic phase have a lower level of ROS production compared with patients in chronic phase (1.23 vs 1.09, p=0.03). Our results of fluorescence 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 JAK2 mutation in MPN patient is associated with a low fluidity of platelet membrane. Association of Hydroxyurea and Anagrelide with low level of fluorescence anisotropy. The fluidity of platelet membrane could be an important parameter which influenced 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 future study and need to be checked any correlation between modification of fluidity membrane production of ROS and expression of microparticles platelet derivate.

**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 hospitalization. However, CVC related venous thrombosis frequency. In pediatric age group exact risk factors for CVC related venous thrombosis have not been shown yet. Rotational thromboelastography (ROTEM®) measures clot formation and stability and can be used to determine the risk of thrombosis. In our study we used ROTEM® to predict the risk of venous thrombosis in children with central venous catheter. The aims of this study were to evaluate the clinical performance of ROTEM® for the prediction of venous thrombosis in children with central venous catheter.

Methods: Study included patients who required CVC insertion due to any reason and who were not on any anticoagulation treatment during the week before the CVC insertion. On the day of CVC insertion clotting time (CT), clot formation time (CFT), maximum clot firmness (MCF), and alpha angle (AA) were measured for intrinsic (INTEM), extrinsic (EXTEM), and fibrinogen (FIBTEM) pathways via ROTEM. At one week of insertion and at removal of CVC, Doppler ultrasound imaging was performed to the vein that catheter was inserted. Results: A total 14 patients were included in the study. Median age was 3.9 years (range 1-17.9 years). Ten (71%) of the patients had jugular or femoral catheter. Median duration until removal of CVC was 15.5 days (7-56). Thrombosis was detected in one patient (7%) at first week of CVC insertion (Patient 10). When the the ROTEM parameters were examined, this patient had lowest CT and highest AA in INTEM, and the highest AA in EXTEM, indicating most pro-coagulant status (Table 1). Also patient 14 had similar AA as patient 10 in INTEM and EXTEM but was not found to develop thrombosis by the 18th day of insertion. However, CVC of that patient wasn’t removed yet. Summary/Conclusions: In this study we reported our preliminary results. We detected thrombosis only in one patient and according to this limited sample size, we may suggest that CT and AA in INTEM, and AA in INTEM to prior insertion of CVC may be predictive for catheter related thrombosis development. Such patients with pro-coagulant findings at ROTEM prior to CVC insertion may need prophylactic anti-coagulation. The results in a larger sample size will be more definitive to make a conclusion.

**E1604 THE POTENTIAL ROLE OF ANTEINEOPLASTIC DRUGS IN THE PREDICTION OF THROMBOTIC RISK IN ONCOLOGIC PATIENTS IN ADDITION TO THE KHORANA SCORE**

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Background: Venous thromboembolism (VTE) is common in patients with cancer. Several risk factors (related with patient, tumour and treatment) have been already identified. Thromboprophylaxis (TP) with low molecular weight heparin (LMWH) is associated with a reduction of symptomatic VTE but without clear benefit in survival as the number of major bleedings is increased. Primary TP in newly diagnosed cancer patients starting chemotherapy (CT), a risk assessment model based on clinical and laboratory variables was developed (the Khorana score). Many patients with intermediate risk (without thromboprophylaxis indication according to Khorana-based clinical guidelines) develop VTE episodes. Factors as tissue factor-bearing micro particles and D-Dimer levels in addition to lenalodimide, platin and gencitabine-based therapies are associated with VTE high risk. Its efficacy as a predictive tool is a matter of debate.

Aims: This retrospective, observational study is aimed to assess the Khorana score efficacy in predicting the VTE risk and analyze some treatment related factors as predictive complementary tools.

Methods: We analyzed the demographic characteristic, the Khorana score and the antineoplastic treatment of oncologic patients diagnosed of pulmonary embolism (PE) from December 2010 until December 2016 at the Complejo Hospitalario de Navarra. At baseline, the Khorana score classified patients as 'low risk' (0 points) intermediate risk' (1-2 points) or 'high risk' (≥3 points) for VTE.

Results: 102 oncologic patients were diagnosed of PE. Patient baseline characteristics are shown 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' (without tromboprophylaxis indication according to Khorana-based clinical guidelines) develop VTE episodes. Factors as tissue factor-bearing micro particles and D-Dimmer levels in addition to lenalodimide, platin and gencitabine-based therapies are associated with VTE high risk.

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 thrombosis-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 antiithrombic prophylaxis in oncologic patients receiving outpatient chemotherapy treatment.
E1605

CLINICAL OUTCOMES AND UTILIZATION OF BLOOD BANK RESOURCES OF PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA (TTP), HEMOLYTIC UREMIC SYNDROME (HUS), AND OTHER MICROANGIOPATHY ASSOCIATED (MAHA) REACTIONS - EXPERIENCE

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Background: TTP, HUS and other thrombotic microangiopathy are rare, complex clinical syndromes which are characterized by thrombocytopenia, microangiopathic haemolytic anaemia, (MAHA) and systemic thrombosis. The introduction of plasma exchange (PEX) has dramatically reduced the mortality of these patients, and has become standard of treatment. Although the clinical outcome of these conditions is heterogenous, with multiple clinical complications and prolonged hospital stay, there is no previously published data to provide 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%), 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 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=3) and Cyclophosphamide (n=2), achieved clinical response with a median of 15 days from the time second line agents were used. The 1 year overall survival of those who received second line treatment compared to patients who responded to only PEX and standard of care was 59% and 80% (p=0.51), respectively.

Summary/Conclusions: The clinical outcome in terms of survival in our cohort is in keeping with that of other registry and cohort (Hovinka 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. Hepatitis E virus is transmitted by oral ingestion of contaminated food or drinking water. In Italy, the virus were firstly detected by indirect test in 1993, and identification of specific antigen was performed in 1995. More recently, a seroepidemiological study performed in the province of Parma showed that 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 hepatitis E virus 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 (85% 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. After the follow up, only the repetitive reactive donor showed a IgM and IgG seroconversion, indicating primary HEV infection. Therefore, we estimated that the risk of receiving a potentially infectious blood unit is of 1:1000 (upper bound of the 95% confidence interval, 1:1700).

Summary/Conclusions: These results confirm the very low risk of hepatitis E virus transmission, although small, is not negligible. The clinical impact of HEV infection among blood recipients remains to be assessed. These data need to be considered when deciding a national policy for preventing HEV transmission.

E1607

SHORT-TERM ADMINISTRATION OF RECOMBINANT HUMAN ERYTHROPOIETIN DECREASES B CELL IN HUMAN PERIPHERAL BLOOD

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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, vascularization action, and promoting blood cell differentiation in addition to stimulation of erythropoiesis as well, whereas there are conflicting results of small cohorts as to its effect on blood immune cells.

Aims: We analyzed peripheral white blood cell subsets in patients who received one bolus administration of recombinant human erythropoietin (rHuEPO) to examine the effect of EPO on human immune system.

Methods: One hundred nineteen autologous blood donors (male/female 62/57) in Gunma University Hospital were enrolled in this study after written informed consent. All the patients had no infections or inflammation. Forty nine patients were treated with rHuEPO (Epoetin alpha or Epoetin beta, 24,000 IU, respectively) once after blood donation because of low hemoglobin concentration and 70 were not treated. Peripheral blood samples were obtained at the time of the first phlebotomy and after 1 week from the same patient. We measured the number of WBC, lymphocytes, myeloid dendritic cells (mDC), plasmacytoid dendritic cells (pDC), CD4+ T cells, CD8+ T cells, Natural killer (NK) cells, B cells, monocytes, and neutrophils of peripheral blood before and after rHuEPO administration by flow cytometry. Absolute number and percentage of lymphocytes in WBC decreased significantly after rHuEPO administration from 1985.0±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=0.04), from 290.6±157.6/µl to 257.4±141.8/µl (p=0.01) and from 298.3±192.4/µl to 239.9±158.2/µl (p<0.01), respectively. Regarding B cell subsets, absolute number of naïve B cell and IgD-CD27- B cell significantly decreased from 171.3±93.5/µl (153.0±64.2/µl) 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 naïve B cell and IgD-CD27- B cell in total normal 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 lymphocytes and B 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.

Aims: This study was retrospectively analyzed for hematologic malignancies and aplastic anemia with bloodless treatments in Soonchunhyang university hospital.

Methods: A retrospective review of medical records was performed of 44 patients with hematologic malignancies and aplastic anemia who requested bloodless medicine from January 2006 to December 2015 at Soonchunhyang university hospital.

Results: Of 44 patients, 48% were men (n=21) and 52% were women (n=23). The median age of the study population at the time of diagnosis was 62 years (range 16-87). Thirteen patients (29.5%) were acute leukemia, 15 (34.1%) patients with non-Hodgkin’s lymphoma (NHL), 2 (4.5%) patients with aplastic anemia (AA), 6 (13.6%) patients with chronic myeloid leukemia (CML), 4 (9%) patients with myelodysplastic syndrome (MDS) and 4 (9%) patients with multiple myeloma (MM). Thirty one patients were treated with chemotherapies and 13 patients were treated with supportive care only. Among 44 patients 27 patients were died. Most common cause of attribution to death was anemia (92.5%). And Chief complaint at death was dyspnea (88%). Median survival of acute leukemia was 1 month (95% CI, 0.4-1.59).

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 etiology. The primary outcome was the response to therapy defined as reaching the Hb level >13 gr/dL or 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 and low risk patients. Of 48 patients, 65% were men (n=31) and 35% were women (n=17). Mean age was 70.4 years, with a male-female ratio of 1:2, and the patients were divided into 2 groups according to the bleeding risk: high risk and low risk patients.

Summary/Conclusions: The main determinants of transfusion requirements are the duration of anemia, the type of transplant and the disease, with myeloid malignancies requiring more transfusions. The establishment of haplo-identical transplantation 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 ENDOPLASMIC RETICULUM STRESS, AUTOPHAGY, AND TIAF LITERIATION
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Background: Patients suffering from Acute lymphoblastic leukaemias (ALLs) harboring t(9;22) genetic abnormality are classified very high risk (tH) ALLs displaying poor clinical outcome irrespective of intensive chemotherapies and tyrosine kinase inhibitor treatment. Development of new adjuvant therapeutics will provide great value. HQ17(3)[10(2),13(E),15(E)-heptadecatetrayl hydroquinone] isolated from the lacquer tree showed potent cytotoxic effect within 24 hours at micromolar concentration on several ALL cell lines, including TKI (imatinib, IM)-resistant Ph+ B-ALL cell line SUP-B15 cells, but spared normal PB leukocytes, and were non-toxic in experimental rats after 28-day HQ17(3) injection. Thus HQ17(3) presents as a potential anti-leukemic agent and serves a model for design anti-leukemic regimen. We previously showed HQ17(3)-induced rapid cell demise, characterized by oxidative stress, loss of membrane integrity, mitochondrial membrane potential disturbance and nuclear DNA fragmentation. Neither pan-caspase inhibitor nor Nec-1 (RIP-1 inhibitor) protected from HQ17(3) induced apoptosis of SUP-B15 cells from HQ17(3)-induced cell death. The cell death program elicited by HQ17(3) is caspase-independent, and is different from the RIP1-mediated controlled necroptosis.

Aims: To investigate the characters of, and the molecular pathways involved in the HQ17(3)-induced non-classical death on VHL-ALL SUP-B15 cells and help design effective therapeutic strategies for the tH-ALLs.

Methods: Cell growth inhibition in response to HQ17(3) w/o inhibitors was analyzed by ACP assay. Cells were stained by Annexin V/PI and analyzed by flow cytometry for cell death. Lysosomal protease inhibitors (AEBSF (serine protease inh.), pepstatin/CA074-Me (cathepsin D/B inh.)) or autophagy inhibitors (3-MA, E64d) were used in combination with HQ17(3) in some experiments. Acidine orange stain and confocal microscopy are used to visualize the changes of acidic vesicles. Autophagic flow in response to HQ17(3) was revealed by aggregation of ectopically expressed EGFP-LC3. Western blot analysis were used to detect protein expression levels.

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 cathepsin release from lysosomal membrane permeabilization (LMP) as cathepsin inhibitors did not attenuate the cell death. HQ17(3) enhanced autophagy as revealed by aggregation of ectopically expressed EGFP-LC3. Inhibition of autophagy by Bafilomycin A1 or knockdown of the essential autophagy-related Beclin 1 by shRNA could partially attenuate HQ17(3)-induced cell death. Further, HQ17(3) treatment gave rise to early ER stress as revealed by enhancement of eIF2α phosphorylation and up-regulation of ER chaperone Grp78. HQ17(3) induced nuclear translocation of AIF, in compatible with mitochrondria disturbance and caspase-independent cell death thereafter.

Summary/Conclusions: In Ph+-ALL SUP-B15 cells, HQ17(3) acts in multifaceted: a) lead to oxidative stress and membrane permeabilization, b) induce ER stress and calcium mobilization to mitochondria, cleave and release AIF to mediate nuclear chromatin cleavage, c) HQ17(3)-induced autophagy may be implicated cell death. This study shows agents that are capable of eliciting an intricate effector network in therapy-induced cytotoxicity will have potential as adjuvants controlling the VHL-Ph+-ALL cell refractory to conventional high dose chemotherapies and TKI regime.

PB1612
TARGETED MUTATIONAL PROFILING OF CHILDHOOD AND ADULT ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS
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Background: Acute lymphoblastic 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 (TSCP) that targets mutational hotspots in 48 cancer related genes (212 ampiclons). The bioinformatic analyses was conducted using processing pipeline composed of both freely available open source bioinformatics tools as well as tools developed in house. The average coverage of high-quality sequences was 2500 x ampli- con. Ten genes were discarded due to insufficient coverage, therefore we ana- lyzed a total of 183 amplicons from 38 genes. Variants were identified in relation to the GRCh37 reference genome by applying a Bayesian approach and com- pared to public genetic variation databases and in-house databases.

Results: We identified a total of 331 (159 cALL, 172 aALL) variants in the coding regions (median per patient: 9, range: 6-12; median per cALL: 9; range: 6-12; median per aALL: 10; range: 7-12) and 429 (211 cALL, 218 aALL) variants in the non-coding regions (median per patient: 13; range: 10-15; median per cALL: 13; range: 10-14; median per aALL: 13; range: 10-13). Overview of 98 variants (median per patient: 2.8, range: 1-6) were potentially protein-chang- ing, including nonsense, frameshift, and missense (NFMI) mutations. There were 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). None of these NFM mutations in STK11, 3 in ABL1, RET KRAS and 2 in HNF1A, NRAS, and NOTCH1. Observed in individual patients detected mutations predominantly disrupted Ras/RKT pathway (STK11, KIT, MET, NRAS, KRAS, PTEN). Additionally, we identified 5 patients with the same mutation in HNF1A gene coding for transcriptional factor, disrupting both Wnt and Notch signaling pathways. HQ17(3) treatment gave rise to early ER stress as revealed by aggregation 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 detect protein expression levels.

Summary/Conclusions: Our targeted NGS study showed low number of recur- rent mutations in both cALL and aALL patients. HQ17(3) revealed a few key signaling pathways, primarily Ras/RKT and Notch pathways. This study has contributed to knowledge of ALL mutational landscape, leading to better under- standing of molecular basis of ALL and better stratification and treatment of ALL patients.
acquired results were compared with those derived from the first ALL diagnosis. Results: The median blasts in BM were 36.9% (range: 2-78%) of BM blasts cells count in 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.

Summary/Conclusions: Multiplex IHC enables facile 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.

PB1614
IMMUNOLOGICAL CHARACTERIZATION OF PH+ ALL BONE MARROW BY MULTIPLEX IMMUNOHISTOCHEMISTRY
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Background: The treatment results in Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL) have improved significantly in the era of tyrosine kinase inhibitors (TKIs). However, many patients relapse despite having received intense treatments with initially favorable responses. TKI therapy is known to modulate the immune system, and it may play a critical role in keeping the leukemia under control. However, little is known about the status of the immune system in patients with Ph+ ALL. Especially with the emerging immunotherapies in sight, it is vital to chart the immunological landmarks that could help us direct the treatment towards a more personalized course.

Aims: To characterize the immunological microenvironment in Ph+ ALL bone marrow (BM) by multiplex immunohistochemistry (IHC).

Methods: Ph+ ALL BM biopsies from the diagnosis stage were collected from Helsinki University Hospital and Tampere University Hospital (N=31). BM biopsies from non-leukemic (NL) controls (N=14) were used as a reference. Samples were hematopathologically evaluated and a tissue microarray (TMA) was constructed by selecting two BM cores with high leukemic cell infiltration per patient. The TMA sections were stained with both fluorescent and chromogenic dyes for six markers and nuclei simultaneously enabling cytometric analysis at cell resolution. Marker panels included T and B lymphoid cells, NK and dendritic cells, macrophages as well as myeloid derived suppressor cells. Furthermore, we analyzed immune checkpoint molecules (PD1, LAG3, OX40, TIM3, CTLA4) and their ligands (PD-L1, PD-L2, HLA-G, HLA-ABC) alongside with various activation markers (granzyme B, CD45RO, CD25, CD57, CD27).

After the staining, the cells were segmented and analyzed using the image analysis software CellProfiler and the cell analysis software FlowJo.

Results: The CD4+/CD8+ ratio was lower in Ph+ ALL BM versus NL BM (1.3 [interquartile range (IQR) 1.0-1.9] vs 2.0 [IQR 1.7-2.4]; P=0.0134) indicating that there are relatively more CD8+ T cells in the leukemia than in the non-leukemic BM sample. The ratio of memory CD4+CD45RO+ T cells in Ph+ ALL BM versus NL BM was elevated (21.0% [IQR 16.7-28.5] vs 13.0% [IQR 8.7-15.9]; CD4+ T cells, P=0.0044). The difference in memory CD8+CD45RO+ T cells was not significant (P=0.36). Further analysis of the T cell phenotype showed increased proportion of both PD1-positive helper T cells and PD1-positive CD8+ T cells in Ph+ ALL BM versus NL BM (29.7% [IQR 17.5-40.1] vs 6.9% [IQR 5.7-8.9]; PD1+ T cells, P=0.0001) and 28.8% [IQR 13.2-38.0] vs 14.9% [IQR 9.6-18.7]; of CD8+ cells, p=0.0107). The ratio of OX40-positive helper T cells was also higher in Ph+ ALL BM (27.1% [IQR 21.6-33.25] vs 18.5% [IQR 14.8-21.9], of CD4+ cells, P<0.0001), but no difference was observed in the proportion of OX40-positive CD8+ T cells (P=0.49).

Summary/Conclusions: Multiplex IHC enables facile 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.
Results: The prevalence of the CDKN2A deletion in all studied population was 24.5% (27 cases). The frequency of homozygous deletions was 70% (in 19 cases), heterozygous deletion was 30% (in 8 cases). CDKN2A deletion was detected in 14 (52%) patients with precursor-B phenotype, in 11 cases (41%) with T-ALL and in 2 (7%) cases with biphenotypical ALL. Our study demonstrated that CDKN2A deletion had no significant association with age, sex, WBC counts, BM blasts, risk stratification groups, complete remission (CR) and relapse rate in B-cell ALL. We didn’t reveal any significant differences in OS, clinical and laboratory dates between groups of patients with homozygous and heterozygous deletion of the CDKN2A deletion. The analysis for T-ALL has detected that CDKN2A deletion was strongly associated with high WBC count (the median is 80(10-200), with high (30%) yield of lymphocytes (linear regression) (the median is 306 E/L, p=0.0004) and no associating with CR and replace incidence was found. We didn’t revealed relationship between CDKN2A deletion and ALL. TEL/AML1 rearrangement, MYC translocation, TP53 mutation and IAMP2. CDKN2A deletion didn’t have statistically significant impact on outcome of patients. The five-year OS for patients with and without deletion was 85% and 76% (p=0, 40); free survival (DFS) was 91% and 71% (p=0, 09), respectively. Results: We were unable to demonstrate prognostic value of the CDKN2A deletion in adult ALL patients and did not find significant associ- ation between deletion of the CDKN2A gene and with known cytokrogenic prognos- tic factors. However patients with T-cell ALL and CDKN2A deletion had a more aggressive clinical course features (high level WBC and LDH), but it didn’t associated with poor outcomes including overall survival. Deletion of CDKN2A is not adverse prognostic factor in adult ALL treated according to protocol RALL-2009.

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 cytokrogenic prognostic factors. However, patients with T-cell ALL and CDKN2A deletion had a more aggressive clinical course 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 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 deletion in 53 patients with enough quality DNA. CDKN2A copy-number (CN) alterations in samples with few or not enough quality DNA (n=53). We corrected our results obtained by the array and/or qPCR were checked by FISH, when samples were available. Cumulative incidence of complete remission in over 90% of the cases. Nevertheless, 1 out of 5 children didn’t attained CR and heterozygous on CDKN2A/B locus mutation was studied on paired samples by Sanger sequencing.

Results: qPCR results showed that most of the 9p21 losses corresponded to homozygous deletions in all cases (36%, 19/53), while heterozygous deletions corresponded to 7.5% (3/53) and different CNA status between CDKN2A and B to 28% (15/53) of the samples. Global alterations in CDKN2A/B locus were present in 30 (61%) of patients. In 9 cases deletions obtained from the arrays corroborate the findings obtained by qPCR. The resolution of the array allowed us to distinguish between homozygosis in CDKN2A and heterozygosis on CDKN2B. The Fisher analysis corroborated the homozygous deletion in the CDKN2AB 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. We analyzed the copy-number (CN) alterations in samples with few or not enough quality DNA.

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. Supported by 2014 SGR225 (CRE) from CERCA Programme/Generalitat de Catalunya, and by funds from Josep Carreras International Foundation, “la Caixa” Foundation and Celgene Spain.

PB1617 BUTEIN KILLS ACUTE LYMPHOBLASTIC LEUKEMIC CELLS IN VITRO AND IN VIVO THROUGH FOXO3A AND CASPASE-DEPENDENT APOTOTIC PATHWAYS

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Background: Acute lymphoblastic leukemia (ALL) is a common hematological malignancy in children. Discovering and developing effective chemotherapeutic drugs are needed for ALL.

Aims: In this study, the anti-leukemic effect and the potential molecular 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 in vitro and xenografts in severe combined immunodeficient (SCID) mice.

Results: We tested the expression of the caspase-9, poly ADP-ribose polymerase (PARP), nuclear Forkhead class box O3a (FOXO3a) and BCL-2 interacting mediator of cell death (BIM) using western blot assay. We established the xenograft mouse model to examine the anti-leukemic effect of butein in vivo. 1,8-Dihydroxybutein was found to significantly induce the cellular apoptosis of ALL cell lines and primary ALL blasts in a dose-dependent manner. It also activated the cleavage of caspase-9 and PARP. We also found that butein promoted 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 of butein, whereas overexpression of FOXO3a enhanced the butein-induced apoptosis. However, overexpression of FOXO3a mutation (C-terminally truncated FOXO3a DNA-binding domain) decreased the apoptosis by butein through decreasing the expression of BIM. Furthermore, treatment with butein was highly efficacious in vivo, with enhanced reduction of tumor burden in a xenograft model of ALL.

Summary/Conclusions: Our results therefore demonstrate the therapeutic potential of butein for ALL via FOXO3a and caspase-dependent apoptotic pathways.

PB1618 GENOMIC LANDSCAPE AT DIAGNOSIS AND RELAPSE IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Childhood acute lymphoblastic leukemia (ALL) is the most common of pediatric malignancies, but intensive chemotherapy now allows to obtain complete remission in over 90% of the cases. Nevertheless, 1 out of 5 children 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 analyzed by Illumina Infinium assay and SNP array (Affymetrix®) performed on cryopreserved cells at diagnosis and relapse investigated copy number alterations (CNA) and loss of heterozygosity (LOH). TP53 mutation was studied on paired samples by Sanger sequencing.

Results: The 8 patients presented mutations 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 9p (comprising CTKF2/2A, PAX5 and JAK2) for 5 patients and 12p (including ETV6) for 3. Stable CNA were observed in the JAK/STAT pathway in 2 patients (JAK2 and LOH in the RAS/MAPK pathway (NRAS) in 1). Using the genetic classification of Moorman et al based on SNP array for 8 genes at diagnosis (IKZF1, CDKN2A/A, PAR 1, BTG1, EBF1, PAX5, ETV6 and RB1), SNP reclassified our patients in 3 of good prognosis and 5 of poor prognosis, with a median of 2 CNA for the 8 genes of interest. The 2 patients with cytogenetic intermediate prognosis would thus probably have been considered for a more intense therapeutic regimen, i.e. allogeneic stem-cell transplantation. Moreover, SNP showed that 2 patients acquired an IKZF1 deletion, also of poor prognosis, while none of the children had TP53 mutation at diagnosis nor relapse.

Summary/Conclusions: SNP array allowed to identify additional anomalies (compared to karyotype) in all children tested and changed the prognostic value of diagnostic anomalies. Moreover, the identification of anomalies in the JAK/STAT pathway could indicate a treatment by tyrosine kinase inhibitors, which would possibly have positively modified outcome. Taken together, this new technology combined with classical analyses at diagnosis might modify therapeutic options in childhood ALL, especially in the subgroup with a normal karyotype.

PB1619
SCREENING OF NUDT15 GENE VARIANTS IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: In cells, while DNA bases can be protected by double helix formation and nucleosome packaging, deoxyribonucleotide triphosphates are unprotected, thus, are vulnerable to damage. One of the enzymes which are responsible for removing damaged nucleotides is Nudix hydrolase15 (NUDT15). NUDT15 works as a negative regulator in thiopurine metabolism. Thioguanines and related compounds are used for treatment of acute lymphoblastic leukemia. NUDT15 inhibition leads to incorrect DNA synthesis and induction of apoptosis. NUDT15 inhibits incorrect base pairing and apoptosis through catalysis of theophylline hydrolysis. Tanaka et al. claimed that, besides TPMT variants in Japanese patients, there might be possible additional factors that may influence thiopurine toxicity. They reported that NUDT15 variants are more specific to Asian population when compared to European people. As far as we know, this is the first study on screening of possible variants in the first exon of NUDT15 in Turkish children with precursor B-cell acute lymphoblastic leukemia (Pre-B ALL).

Aims: In this study, we aim to 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 diagnosed with Pre-B ALL at Lüskante Hospital. DNA samples were isolated by using MagNa Pure isolation 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+53insGGGGGC>c) in 10 patients (12%). The other intronic variation was defined as c.79670700 (c.158+117C>T), rs3831098 was determined in one of the 83 patients and rs7967000 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).

PB1620
COMPREHENSIVE MOLECULAR CYTOGENETIC ANALYSES OF BONE MARROW CELLS IN 64 CHILDREN WITH T-ALL REVEALED PROGNOSTICALLY RELEVANT RECURRENT FINDINGS
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Background: T-ALL represents 15% of newly diagnosed children with ALL and it is a clinically and genetically heterogeneous disease. Despite the use of intensive chemotherapy, relapse occurs in almost 25% of patients whose outcome remains dismal. Visible chromosomal aberrations are seen in approximately half of the cases, while cytogenetically cryptic aberrations are observed in almost all cases of T-ALL. However, prognostic implication of majority of them still remains unclear.

Aims: The aim of this study was to retrospectively and prospectively analyze bone marrow cells of children with T-ALL, to determine a frequency of recurrent cryptic chromosomal aberrations and to assess their impact on event free survival (EFS) and overall survival (OS).

Methods: Bone marrow cells of all patients were analyzed at the time of diagnosis by combination of conventional and molecular cytogenetic methods. For detection of the most frequent known chromosomal changes, i.e. rearrangements of TRC loci (TRA-14q11, TRG-7q34, TRG-7q14) and TLX3 gene (6q25), deletion of CDKN2A (9p21) and amplification of ABL1 (9q34), interphase FISH with locus-specific probes (Dako, Abbott Molecular) was used. Complex chromosomal rearrangements were proved by multicolor FISH and multicolor banding (24XCyte/XCyte Probe Kit; MetaSystems) or CGH SNP array (SurePrint G3 CGH+SNP 4x180K slide, Agilent). For OS and EFS Kaplan-Meier analysis with Mantel Cox test was done.

Results: During the years 1996-2016 we examined archived material of 64 children with T-ALL (19 girls and 45 boys, median age 8.25 years). In total, 145 chromosomal aberrations were detected in 86% of patients. The most frequent aberration was deletion of CDKN2A gene, which was found in 35/64 patients (19x homozygous, 16x heterozygous). Rearrangements of TCR loci were detected in 17/64 children (11x TRA, 6x TRB). TLX3 gene rearrangement was established in 15/64 patients. No aberration of TRG gene and amplification of ABL1 were found. Complex chromosomal aberrations were proved in 12/64 children. In two cases, isochromosome of the long arm of chromosome 9 was found. 48 patients are living in the first/second complete remission. Relapse of the disease occurred in 17 patients, 16 children died. Best outcome (EFS and OS) was associated with TRA translocations (p<0.05). Patients with TLX3 rearrangement had significantly shorter OS and EFS (p<0.05).

Summary/Conclusions: Using molecular cytogenetic methods cryptic recurrent aberrations were proved in vast majority of patients. Rearrangement of TLX3 gene was related to poor outcome in contrast to TRA translocations associated with more favorable course of the disease. Our work attempts to clear up the significance of chromosomal aberrations related to childhood T-ALL in order to facilitate the patients’ stratification into cytogenetic prognostic groups and to identify patients at an increased risk of relapse similarly like it has been adopted in B-ALL.

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PB1621
ADULT PRIMARY ACUTE LEUKEMIA SAMPLES WITH CHROMOSOMAL TRANSLocations GROW WELL IN IMMUNODEfICIENT MICE, BUT ARE DifFICULT TO TRANSDUCE WITH LENTIVIRUSES
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Background: Acute leukemia (AL) is a severe disease of the hematopoietic system and associated with a poor outcome for patients. Patient derived xenograft (PDX) mouse models provide an attractive tool to engrat and grow primary tumor cells. In contrast to culture growth, samples can be monitored in a consistently
microenvironment. This powerful tool provides the baseline for further experiments like preclinical treatment trials or biology studies. While good engraftment rates were published for primary pediatric ALL samples, engraftment rates of adult ALL samples might be inferior, but remain largely elusive.

Aims: This study aimed to determine engraftment and growing ability of primary adult 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 samples were transplanted into NSG mice in the absence of total body irradiation. Both frozen as well as fresh patient material was used. Human CD45 and human CD38 were stained in blood to monitor successful engraftment. Mice were sacrificed before coming down with leukemia. Isolated cells from bone marrow and spleen were analyzed by flow cytometry. Genetic engineering was performed using lentiviral vector systems and monitored by expression of fluorochrome markers and flow cytometry.

Results: Engraftment and growth was successful in NSG mice in 12 out of 15 primary adult ALL samples. Frozen samples showed a longer median engraftment time than fresh samples. fresh samples could already be transplanted 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 fluorochrome colours for cell marking and monitoring in further experiments. Lentiviral transduction was performed in 8 ALL samples with BCR-ABL rearrangement and 2 ALL-44 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 in contrast to ALL, transduction efficiency for AML rearranged samples can be transduced with lentiviruses with identical high transduction efficiency as pediatric samples, with an age independent exception of ALL PDX cells with BCR-ABL or MLL translocations.

PB1622
SYNERGIC CHEMOTHERAPEUTIC EFFECT OF MENADIONE COMBINED WITH EPIGALLOCATECHINE-3-GALLATE OR DOXORUBICIN IN A HUMAN CELLULAR MODEL FOR ACUTE LYMPHOCYTIC LEUKEMIA
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Background: Epigallocatechine-3-gallate (EGCG) and menadione (vitamin K3; MD) are known as potent apoptotigens in cellular models for acute lymphocytic leukemia (ALL) – Jurkat T cells.

Aims: The goal of this study was to explore the chemotherapeutic potential of MD combined with EGCG or DOX, and to determine whether there is a synergic interaction between these agents that could significantly enhance their antitumoral effect in a cellular model of ALL. We investigated the antiproliferative effect of MD combined alone or in combination EGCG:MD and MD: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 spectrofluorimetry, using the fluorescent probes CM-H2DCFDA and JC-1, respectively.

Results: Clonogenic survival was 117 ± 17 µM (Hill coefficient n=3.17) and mitochondrial calcium in a dose-dependent manner (IC50 = 97 ± 3.2 mM, n=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 speacies (r=−0.437) production, thus EGCG exerted a depolarization 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 LEUKEMIA
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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]. Focal deletions of exons 3-9 were a second cause for short ERG variants. Up to 7% of pre-B ALL showed deregulated expression of both genes, DUX4 and ERG [2].

Aims: We set out to find pre-B-ALL cell lines with DUX4 translocation and ERG deletion as potential model systems for this novel subtype of pre-B-ALL.

Methods: We screened a panel of ALL cell lines for aberrant expression of DUX4 and MD as potential models for this novel subtype of pre-B-ALL.

Results: Genomic PCR was performed to detect focal ERG deletions, qRT-PCR showed expression of alternative ERG exon 6, transcriptional target of DUX4.

Summary/Conclusions: In conclusion, focal ERG deletions in pre-B-ALL cell lines (2/66) occur at similar frequencies as in the primary tumor. Cell line NALM-6 carries the DUX4-IGH translocation, expresses the DUX4 protein and an ERG mRNA variant including the alternative exon 6. ERG deletions were present in cell lines NALM-6 and SUP-B15. However, cell line SUP-B15 did not express DUX4 protein and consequently also not alternative ERG exon 6 transcript. These results indicate that focal ERG deletions are not a safe indicator for aberrant expression of DUX4. Cell line NALM-6 is presented as model system for DUX4/ERG pre-B-ALL.

References
2 Zhang J, McCastlain K, Yindath F. DUX4 translocations. Genomic PCR was performed to detect focal ERG deletions, qRT-PCR showed expression of alternative ERG exon 6, transcriptional target of DUX4.

PB1624
NATURAL HISTORY OF SECONDARY MULTILINEAGE PROLIFERATION WITH MONOSOMY 7 FOLLOWING TREATMENT OF RELAPSED ACUTE LYMPHOCYTIC LEUKEMIA
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Background: Approximately 90% of children with acute lymphoblastic leukemia (ALL) are cured with current treatment protocols. However, 15-20% of the patients still experience disease relapse. Moreover, small subset of 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 followed by aberrant proliferation of several different subsets of precursor cells in bone marrow (BM), which was associated with progressive ineffective hematopoiesis.

Methods: A boy diagnosed with standard risk B-cell Precursor (BCP) ALL in 10-2009 was treated until 12-2011 with frontline chemotherapy according to ALL-IC BFM 2002 protocol. In 12-2012, one year after treatment completion, he relapsed with BCP-ALL, BM blasts with monosomal karyotype was performed, while the biopsy of the second tests showed no leukemic infiltration. He received 2nd line chemotherapy according to InTReALL 2010 and local radiotherapy for the testicular area. Despite the borderline minimal residual...
Results: BM aspirate morphology showed 5% of blasts. However, detailed 8- channel flow cytometry analysis revealed polyclonal pattern in all BCP subsets. Cytogenetic analysis showed an altered 45.XY.del(4)(q31.3), t(7;9)(200kb). In addition, several gains of minor chromosomal regions were detected in CD34+ (90.0%), CD34+ (18.7%), CD34+ (90.0%), and CD34+ (18.7%), CD34+ (90.0%). Due to progressive increase of all subsets of abnormal precursor cells (27.5% in total) and hematopoietic dysregulation, further treatment direction was set at haploidentical stem cell transplantation.

Summary/Conclusions: We present an abnormal secondary proliferation, with increased numbers of aberrant BCP, myeloid and plasmacytoid dendritic cell precursors resulting from stem cell defect hallmarked by monosomy 7.

PB1625
IDENTIFICATION OF KEY GENES AND CONSTRUCTION OF MICRORNA−RNA 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 (BMNCs) 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 necrosis were assessed by western blot. The effect of chidamide on NF-κB signaling pathway activity and expression of key molecules when inducing necrosis 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 western blot in APL cell lines.

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 is higher in tumor 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 significantly exert its effect of inducing cell death by inducing necrosis. Chidamide inhibits the translation and translation to c-FLIP, 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 mixed lineage kinase domain-like (MLKL) showed a significant increase.

Summary/Conclusions: c-FLIP, mRNA expression level is abnormally high in T-ALL patients both at initial presentation and at relapse. The expression level of c-FLIP, 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, could be used as a diagnostic marker in T-ALL. Chidamide suppresses histone deacetylation 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, 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 LYMPHBLASTIC 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 the high survival rates (higher than 80%), a considerable 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 CYP1A1 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 xenobiotic metabolizing enzyme that activates the conversion of environmental chemicals into carcinogens. The above gene contains two important single nucleotide polymorphisms, CYP1A1*2A (rs4646903) 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) and 50  adults  blood donors) as control group. Genomic DNA was isolated from peripheral blood of participants and was analyzed for the existence of polymorphisms in CXCL12 loci, the frequencies of AA, AG and GG genotype were 3.45%, 93.1% and 3.45% in children with ALL, 13.3%, 60.0%, 26.7% in children control group and 4.17%, 45.83% and 50.0% in adult control group respectively. In the CYP1A1 loci, the frequencies of AA, AG, and GG genotype were 13.3%, 86.7% and 0% in children with ALL, 90.0%, 10.0%, 0% in children control group and 81.6%, 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 polymorphism and ALL [OR: 37.7 (95% CI: 10.81, 131.37), p<0.001 and OR: 58.5 (95% CI: 9.66, 354.12), p<0.001 using only the children’s control group].

Summary/Conclusions: A higher frequency of CYP1A1 heterozygote allele was observed among children with ALL compared to controls, whereas no differences were observed regarding CXCL12 polymorphisms. Future studies in larger populations are needed in order to specify the role of the above polymorphism in childhood ALL.

PB1627
INTRACHROMOSOMAL AMPLIFICATION OF CHROMOSOME 21 IN PEDIATRIC ACUTE LYMPHBLASTIC LEUKEMIA: A RARE SUBTYPE
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Despite the high survival rates (more than 80%), a noteworthy number of childhood leukemia and represents one third of all pediatric malignancies. In the CXCL12 loci, the frequencies of AA, AG and GG genotype were 3.45%, 93.1% and 3.45% in children with ALL, 13.3%, 60.0%, 26.7% in children control group and 4.17%, 45.83% and 50.0% in adult control group respectively. In the CYP1A1 loci, the frequencies of AA, AG, and GG genotype were 13.3%, 86.7% and 0% in children with ALL, 90.0%, 10.0%, 0% in children control group and 81.6%, 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 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.
Aims: To assess the frequency as well as the clinicopathological and genetic characteristics of ALL with iAMP21 in one of the three national diagnostic centers of pALL in Hungary. We sought to determine additional genetic aberrations associated with this rare entity.

Methods: Between 2008-2016, 175 samples of pALL patients were tested with FISH for BCR-ABL1, ETV6-RUNX1 and MLL translocations. When available, bone marrow karyotyping was used to verify the abnormal results. In one case with iAMP21, multiplex ligation-dependent probe amplification (MLPA) was used to verify the cytogenetic aberrations as well as to detect associated copy number alterations.

Results: Among the 175 samples screened with FISH, three showed evidence of iAMP21 (1.7%). Case 1 was a 16-year-old male who presented with thromocytopenia 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 normal bone marrow cells. The patient 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 in over 4 cases. Observing BCR-ABL1 translocation in a subpopulation of leukemic cells is an intriguing phenomenon; it indicates that this translocation may occur as a secondary event even after leukemic transformation has commenced.
Acute lymphoblastic leukemia - Clinical

PB1629

COMPLETE REMISSION WITH BLINATUMOMAB IN TWO PATIENTS WITH SKIN RELAPSED B-CELL ACUTE LEUKEMIA

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Background: Blinatumomab is a bispecific T cell–engager (BiTE) antibody (CD19/CD3) indicated in relapsed/refractory B-cell Acute Lymphoblastic Leukemia (r/r ALL) (Topp et al.). Extra-medullary relapse is a rare event occurring in only 8% of the patients, of whom only 1.4% present a skin relapse which harbor a dismal prognosis (Gokbuget et al.).

Aims: Herein, we report the efficacy of Blinatumomab in two patients presenting with extra-medullary relapse of ALL.

Methods: The first patient (a 40-year-old man) was diagnosed a CD19+ Ph - B-ALL in 2009. He received a chemotherapy regimen according to the GRAALL protocol (Huguet et al.) until complete remission (CR). In 2015, he presented with a maculopapular rash of the right leg and the left flank, and two enlarged inguinal lymph nodes. Cutaneous relapse was attested by examination of skin biopsy specimen showing a blastic dermal infiltration harboring a CD10+, Tdt+ phenotype. The second patient was a 50-year-old male who presented, in 2016, a CD19+ B-ALL Ph- &Ikaros- without central nervous system involvement. He obtained a first CR after GRAALL induction with negative MRD (IgH) but he relapsed 3 months later with a maculopapular rash of his chest. The skin biopsy revealed a blastic dermal infiltration. These two patients with skin relapse received a short course of the chemotherapy (COBRAALL 2007 regimen) (Domench et al.), with no efficacy (cutaneous blastic infiltrate). Both patients received one cycle of Blinatumomab from day 1 to day 28, at 28 µg per day, in an attempt to achieve CR before allogeneic stem cell transplantation, as previously described.

Results: At day 5 of Blinatumomab, an important non pruritic maculo-papular rash occurred in both patient, in the same area of the initial cutaneous involvement. Interestingly, it decreased after day 8. No new drug introduction or infection (bacterial, viral or parasitic) was documented in the days preceding or during Blinatumomab infusion. A skin biopsy performed at day 6 of Blinatumomab showed an intradermal CD3+ lymphocytic infiltrate with a perivascular, but also a peri-nervous distribution (on the first patient's specimen only). Few lymphocytes marginalized at the basement membrane and rare basophilic keratinocytes were also noted but without blast for the first, although few residual blast cells were observed on the second's. One month later, another skin biopsy showed a CR with lymphocytic infiltrate. The medullar CR was confirmed at the molecular level (MRD negative). The first patient received allogeneic stem cell transplantation (SCT) from a matched related donor one month later. He presented an acute and chronic GVHD, and is now in complete remission with a follow-up of 7 months. The second is still waiting for a SCT.

Summary/Conclusions: These observations confirm the strong efficacy of Blinatumomab in r/r B-ALL. We observed a T-cell dermal recruitment 6 days after Blinatumomab initiation clinically mimicking skin GVHD. However, we couldn’t find specific histological features of GVHD, but only an “inflammatory pseudocyst” of Blinatumomab with a cutaneous infiltration suggesting promising activity in extra-medullary relapse. Further studies are required to confirm a Blinatumomab-based strategy in extramedullary relapsed B-ALL. This may provide a better understanding of how cytolytic synapses between T lymphocytes and intradermal blasts happen and the underlying homing mechanisms involved.

PB1630

A NOVEL METHOD FOR MINIMAL RESIDUAL DISEASE ANALYSIS IN PHILADELPHIA-NEGATIVE ACUTE LYMPHOCYTIC LEUKEMIA: MODIFIED BIOMED-2 POLYMERASE CHAIN REACTION FOR IMMUNOGLOBULIN HEAVY CHAIN REARRANGEMENT

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Background: Recent studies have demonstrated the clinical importance of minimal residual disease (MRD) monitoring in adult acute lymphoblastic leukemia (ALL) as well as pediatric ALL. However, patient-specific polymerase chain reaction (PCR)-based MRD assessment, one of the most commonly recognized methods, is not widely used in clinical practice because it is expensive, time consuming, and technically difficult. Therefore, we modified the BIOMED-2 protocol, PCR for immunoglobulin heavy chain (IgH) rearrangement, to assess MRD in ALL easily and readily in our hospital.

Aims: The aim of this study was to examine the clinical utility of monitoring MRD by the modified BIOMED-2 PCR for IgH rearrangement in patients with Philadelphia-negative (Ph -) ALL.

Methods: We enrolled 54 patients diagnosed with Ph - ALL between 2006 and 2016 in our hospital. IgH rearrangement was detected in 35 patients using the standard BIOMED-2 PCR protocol. Patients who received palliative chemotherapy, never achieved remission (blasts 5%), or had no follow-up MRD data were excluded. Finally, data from 27 patients with Ph - ALL were analyzed. We assessed MRD with the modified BIOMED-2 PCR for IgH using bone marrow samples collected after each chemotherapy session. Patients’ MRD statuses were classified as follows: Early MRDneg, achievement of MRD negativity within 6 weeks after chemotherapy initiation; Late MRDneg, achievement of MRD negativity more than 6 weeks after chemotherapy initiation; or MRDpos, persistent MRD detection during chemotherapy. The endpoint was disease-free survival (DFS), calculated from the date of achieving remission.

Results: The median age was 38 years (16–73), and the median follow-up time was 47 months (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.

<table>
<thead>
<tr>
<th>MRD Status</th>
<th>Early MRDneg</th>
<th>Late MRDneg</th>
<th>Late MRDpos</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Patients</td>
<td>8</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Sex</td>
<td>M, 6; F, 2</td>
<td>M, 12; F, 2</td>
<td>M, 4; F, 1</td>
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<tr>
<td>Age (years)</td>
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<td>38</td>
<td>38</td>
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<tr>
<td>MRDneg Achievement</td>
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<td>After 6 weeks</td>
<td>After 6 weeks</td>
</tr>
<tr>
<td>DFS (months)</td>
<td>47</td>
<td>47</td>
<td>47</td>
</tr>
</tbody>
</table>

* WBC risk: B >3 × 104/μL, T >10 × 104/μL.
** Cytogenetic risk: Hypodiploidy, complex karyotype, MLL rearrangement.
*** Achievement of remission after 2 cycles of chemotherapy.

MRD, minimal residual disease; PCR, polymerase chain reaction; IgH, immunoglobulin heavy chain; M, male; F, female; WBC, white blood cell; CR, complete remission.

Summary/Conclusions: The modified BIOMED-2 PCR protocol is 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).

Figure 1. The status of minimal residual disease was associated with prognosis.

Summary/Conclusions: The modified BIOMED-2 PCR protocol is a highly accurate and reliable method of MRD assessment in adult ALL. It predicted treatment outcomes in adult Ph - ALL, and patients with late MRDneg might derive a high survival benefit from allogeneic transplantation. Finally, the accuracy and reliability of the modified BIOMED-2 PCR for IgH were confirmed with a comparison to quantitative real-time PCR for BCR-ABL using samples from patients with Philadelphia-positive ALL (data not shown).

PB1631

SYSTEMATIC LITERATURE REVIEW OF PEGASPARGASE FOR THE TREATMENT OF NEWLY DIAGNOSED ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Asparaginase is a component of a multi-agent chemotherapy regimen widely used in clinical practice for treatment of acute lymphoblastic leukemia (ALL). Since 2006, pegaspargase (PEG-ASP) has been the gold standard asparaginase for the treatment of pediatric ALL as it offers equivalent efficacy to native E. coli L-asparaginase (native ASP), with less frequent dosing,
an IV administration option, and improved immunogenicity. Clinical outcomes in the adult ALL population are less well understood.

Aims: To assess the relative clinical benefit of PEG-ASP vs native ASP in 1st line treatment in newly diagnosed adult ALL patients in terms of event-free survival (EFS) and overall survival (OS). Safety outcomes were also examined.

Methods: A systematic literature search was conducted using a standardized search algorithm within the National Library of Medicine 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; 77.7]) 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 P. Minetto1,*, N. Bisso1, F. Guolo1, M. Clavio1, E. Covelli1, D. Guardo1, N. Di Felice1, F. Canale1, L. Manconi1, F. Ballerini1, M. Miglino1, R. M. Lemoli1, M. Gobbi1

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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 the present 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 epiphagia, a cervical 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. Steroids therapy determined a borderline increase in toxicity risk (p=0.068, HR 1.25). No increase in toxicity was observed with any dosing of daunorubicin, cyclophosphamide, cytarabine, methotrexate and 6-mercaptopurine (Table 1). Among concomitant antibiotic therapies, vancomycin administration seemed to increase the incidence of grade III/IV hepato-toxicity (p=0.02, HR 1.863). No significant increase was observed with carbapenens and azoles (Table 2). When receiving PEG-ASP with active leukemia, patients in the HSCT cohort remained relatively stable, while the non-HSCT cohort had only half the patients left by the third quarter post relapse. Costs were considered from the perspective of the German statutory health insurance and included costs for prescription medicine as well as outpatient and inpatient healthcare encounters.

Results: Of the total 116 incident adult ALL patients identified, 29 (25%) were determined to have had a relapse and 11 underwent HSCT after relapse (38%). Patients with an HSCT appear to incur higher cost than those without HSCT in each of the quarters after relapse was diagnosed (Table 1), with the highest in the first quarter after relapse, but decreasing in subsequent quarters. Inpatient cost accounted for the majority of the cost for the first three quarters for both HSCT and non-HSCT patients, but more for HSCT patients. The number of patients in the HSCT cohort remained relatively stable, while the non-HSCT cohort had only half the patients left by the third quarter post relapse.

Summary/Conclusions: Our data show that the toxicity profile of PEG-ASP in adult patients is overall manageable. However, serious warnings emerge from our experience. Concomitant drugs and their timing of administration may play a crucial role in significantly contributing to PEG-ASP hepatic toxicity. In order to attempt to reduce toxicity, anthracyclines with shorter half-life, i.e. daunorubicin instead of idarubicin, should be used. A particular attention should be paid when administration of concomitant antibiotic therapy is required.
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 patients and Cyto genetic 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 tolerate the treatment, and 4.7% received a stem cell transplant (AlloHCT). 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 favorable risk factor for OS and LFS of R/R ALL. For relapsed patients, OS was significantly better with first CR duration>6 month and time to transplants 2 months.

Summary/Conclusions: Allo-HCTC is an effective salvage treatment option for patients with refractory and relapsed ALL. Our retrospective analysis showed that R/R ALL with different status prior transplant had similar outcome post transplantation.

PB1635

IMPACT OF DISEASE STATUS ON OUTCOMES OF ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH REFRACTORY AND RELAPSED ACUTE LYMPHOBластIC LEUKEMI

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Background: Refractory or relapse remains a major obstacle in improving outcomes of patients with acute lymphoblastic leukemia (ALL) and allogeneic hematopoietic stem cell transplant (allo-HSCT) was the only curative treatment option for these patients. However, whether an allo-HSCT was performed in status of advanced stage or in setting of remission after salvage chemotherapy, there is no standard of care.

Aims: To evaluate the impact of disease status on the outcomes of allo-HSCT in the treatment of patients with refractory and relapsed ALL.

Methods: 52 patients with refractory and relapsed ALL, including 19 cases in advanced stage (nonremission, NR) and 33 cases in more than or equal to second complete remission (cCR2), received allo-HSCT after myeloablative conditioning regimen in our department.

Results: 51 patients engrafted successfully. The transplantation-related mor-tality (TRM) rate of NR and cCR2 was 10.5% vs 12.1% (P=0.815). The inci-dence of aGVHD was 52.6% vs 57.6% (P=0.730), including 42.1% vs 33.3% (P=0.527) with mild (grade I-II) and 10.5% vs 24.3% (P=0.399) with severe (grade III-IV) aGVHD. The incidence of cGVHD was similar also (41.6% vs 57.9%, P=0.660). With a median follow-up of 12(1.8-44.5) months, the cumulative relapse rate of NR and cCR2 was 47% vs 34.3% (P=0.426) respectively. The estimated 2 year overall survival (OS) and 2 year leukemia-free survival (LFS) rate were 42.6% vs 45.7% (P=0.487) and 46.3% vs 46.2% (P=0.571) respectively. Multivariate Analysis results showed that cGVHD was independ-ently favorable risk factor for OS and LFS of R/R ALL. For relapsed patients, OS was significantly better with first CR duration>6 month and time to transplants months.

Summary/Conclusions: Allo-HSCT is an effective salvage treatment option for patients with refractory and relapsed ALL. Our retrospective analysis showed that R/R ALL with different status prior transplant had similar outcome post transplantation.

PB1636

THE FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF IKZF1 DELETIONS IN ADULT PH-POSITIVE AND PH-NEGATIVE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED IN RUSSIAN ACUTE LYMPHOBLASTIC LEUKEMIA STUDIES

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Background: The incidence of IKZF1 gene deletions is approximately 20% in adult patients with BCR-ABL1-negative B-cell ALL and 70–80% in BCR-ABL1-positive ALL. These mutations are associated with poor prognosis in patients with Ph-positive ALL, but not in patients with Ph-negative ALL, suggesting that other confounding factors may be more prognostically valuable in patients with Ph-neg-ative 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 patients (median age 27, range 17-56; m:f=15:21) with newly diagnosed BCR-ABL1-negative B-cell ALL and 15 patients (median age 34 years, range 22-68; m:f=6:9) with BCR-ABL1-positive B-cell ALL, who were enrolled in Russian acute lymphoblastic leukemia (RALL) - 2009 [ClinicalTrials.gov public site; NCT01193933] and RALL-2012 protocols since Feb 2010 till Sep 2016 and Aug 2009 till Feb 2017, respectively. Intragenic deletions of IKZF1 were detected using breakpoint-specific fluorescent cent multiplex polymerase chain reaction according to the procedure described by [Aurelie Caye et al, Haematologica, 2013]. DNA for PCR was extracted from leukemia cells of frozen bone marrow samples.

Results: The IKZF1 deletions were detected in 7 (47%) of 15 patients with BCR-ABL1-positive B-cell ALL (3 cases with del 4-7 (43%), 2 – del 2-7 (28%), 1 – del 2a-8 and 1 – del 4-8 (14%)). The median follow-up time in 15 patients was 18 months (range: 4-79 month). Five patients died (33%) after relapse or progression of the disease, and 10 patients are alive. Overall survival (OS) for BCR-ABL1-positive 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-negative 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 2-8 (12.5%) and 1 in patient all types of deletions were determined (del 7, del 4-8, del 2-7, del 2-8)). 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-negative 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-positive and with BCR-ABL1-negative ALL was 47% and 22%, respectively. IKZF1 mutations seemed to be of poor prognosis for BCR-ABL1-positive ALL patients.

Correction notice: Fig. 1b - the right panels were corrected.
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. Comparing 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, 9 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 group. 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 MDRT 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. This finding may be related to several aspects: socioeconomic origins, inadequate supportive care for more intensive therapies and ineffective cancer care network. Future prospective studies should focus on this issues.

Figure 1. Relapse-free survival.

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, corticosteroids exposure, poor nutrition, low vitamin D levels, poor muscle mass, genetic predispositions contribute to the development or worsening of bone pathologies during therapy that may result in osteoporosis, fracture and osteonecrosis.

Aims: In this study, we aimed to investigate whether vitamin D receptor and collagen protein gene polymorphisms, which are important in bone mineral and matrix formation, have effects on bone turnover in patients with ALL.

Methods: Fifty children with ALL who were diagnosed and treated with BFM-95 protocol (25 girls, 25 boys) between 1998-2008 and 96 healthy children at Dokuz Eylül University Medical School were enrolled in this study. Polymorphisms of vitamin D receptor (VDR) Fok1 gene and the collagen Col1A1 gene were studied from peripheral blood samples of the patients that were collected before initiation of chemotherapy protocol. After genomic DNA extraction, VDR Fok1 gene and collagen Col1A1 gene polymorphisms were analyzed by polymerase 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, as the number of survivors increased. Bone infiltration of leukemic cells, corticosteroids exposure, poor nutrition, low vitamin D levels, poor muscle mass, genetic predispositions contribute to the development or worsening of bone pathologies during therapy that may result in osteoporosis, fracture and osteonecrosis.

Results: Thirty five patients were included, 21 of them started BFM-based treatment and 14 started GMALL-based protocol. During the first three months, 9 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 group. 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 MDRT 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. This finding may be related to several aspects: socioeconomic origins, inadequate supportive care for more intensive therapies and ineffective cancer care network. Future prospective studies should focus on this issues.
OUTCOME OF ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PEDIATRIC PROTOCOL: MONOCENTRIC STUDY

Background: Several retrospective studies have confirmed that adolescents and young adults (AYA) with acute lymphoblastic leukemia (ALL) treated with pediatric protocols have better outcomes than similarly aged patients treated with adult protocols.

Aims: We reported results and feasibility of a pediatric-based protocol (EORTC 58951) in adolescents and young adults.

Methods: From January 2000 to December 2015, 72 patients aged 16 to 30 years with newly diagnosed ALL were treated, in the department of clinical hematology of Hedi Chaker Hospital, according to the pediatric protocol EORTC 58951. Further leukemia characteristics (Sex, White Blood cell count, Blasts phenotype, Cytogenetic results), we studied the protocol results: response to therapy and prognostic in adolescent and young adults. The majority of the patients (8 patients, 6 at the Intermediate Risk group and 2 at the High Risk group) experienced disturbances during the reinduction 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 reinduction (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 adolescents and young adults receiving therapy for ALL. Awareness of this complication, appropriate parental education for identifying early signs, and prompt therapeutic interventions are essential for optimal outcome. Further studies are required for identifying patients at risk and best use of chemotherapeutic agents and of dexamethasone.
Department of Clinical Institute Fundenuri during 2010-2017 and received chemotherapy according to protocol ALL BFM 1985 and ALL BFM 2002, established after framing in the risk group.

Results: Over a period of 8 years in our department 280 patients with ALL received L-asparaginase in the induction phase. Neurological manifestation suggestive for bleeding or thrombotic events occurred in 9280 (3.21%) patients. 2 patients who were treated according to protocol ALL BFM 1995 and 7 patients were treated according to protocol ALL BFM 2002. M/F ratio was 4/5. Patients had at diagnosis between 3 and 15 years (median age 9 years). All patients had thrombotic events after staring 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 (computerized tomography or magnetic resonance imaging) documented CVST after developing neurological symptoms; one of the patients suffered major complication (extended brain injury) and died. All patients with ALL and thrombotic events received low-molecular weight heparin (LMWH) for 3 to 6 months. A follow-up CT or MRI at 3 to 6 months after diagnosis was made to assess for recanalization of the occluded cortical vein/sinus. Survival in the patients with CVST was 84.61%. 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 II, 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, duration of thrombosis, from headaches, seizures or focal neurological deficits. Severe sepsis association was an increased risk factor for thrombosis 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: Leukemia is the most common cancer in children. Childhood leukemia incidence and survival varies globally, and this could be associated with different demographic risk factors, genetics, and improvement in diagnosis and treatment. Armenia is considered to be a multi-ethnic nation.

Aims: We aimed to quantify the incidence of and mortality from acute leukemias among children population in Armenia and their variation with gender, age, year of diagnosis.

Methods: In this work we included children diagnosed with de novo acute leukemia, 0–18 years of age from 2006 to 2016. The initial data for this survey have been derived from ambulance/dispensary cards, hospitalization journals, and clinical data from the Registry of Blood Diseases at the R. Yeolyan Hematology Center, Yerevan, Armenia. The data has been supplemented by the data from the Registry of Oncological Diseases of the V. Fanaryan NCO, as well as from death certificates. The demographic data has been obtained from the National Statistics Board of Republic of Armenia. The obtained data has been statistically analyzed using EPI INFO-2002 program.

Results: A total of 277 cases of childhood acute leukemia were identified, 174 (62%) were male. The overall incidence of leukemia 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 registred regions in Armenia—Lori, Vayots Dzor and Tavush with higher incidence rate (respectively 2.4, 2.4 и 2.9).

Summary/Conclusions: The increase in the incidence of leukemia in Armenia may be attributed to environmental risk factors, genetics, and improvement in diagnosis and treatment. The increased incidence may be a result of improved diagnostic methods.

PB1644

LONG-TERM SURVIVAL OUTCOMES OF ADULT PHILADELPHIA CHROMOSOME-POSITIVE ACUTE LYMPHOCYTIC 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 no TKI). Thirty-eight percent (n=13) of patients in imatinib group and 13% (n=1) in dasatinib group switched to a different TKI due to adverse effects or failure to achieve remission. There was a trend towards increased 2-year LFS for patients on dasatinib (HR 0.40, 95% CI: 0.14-1.14, p=0.09) and no difference in 2-year mortality (HR 1.00 95%CI: 0.46-2.17, p=0.99). 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).

Summary/Conclusions: The results of this study suggest that dasatinib is superior to imatinib in Ph+ adult ALL, resulting in improved 2-year LFS and OS. Dasatinib is a suitable first-line agent with similar OS and better LFS compared to imatinib.

PB1645

OUTCOME OF ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN A MIXED COHORT OF PEDIATRIC AND ADULT PATIENTS WITH KMT2A-AFF1 ACUTE LYMPHOCYTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) with the use of tyrosine kinase inhibitors (TKIs) along with chemotherapy. However, there are very few studies that describe the comparative effectiveness of various TKIs in this patient population.

Aims: To characterize long-term survival outcomes including leukemia-free survival (LFS) and overall survival (OS) for Ph+ adult ALL patients treated with imatinib versus dasatinib.

Methods: Retrospective chart review was conducted at our institution. Patients >= 18 years old and diagnosed with Ph+ ALL between 2002 and 2015 were included. Analysis was done by intent to treat for patients initiated with imatinib or dasatinib at the time of Ph+ diagnosis. The primary endpoints were 2-year LFS and OS and secondary endpoints were complete molecular response (CMR; BCR-ABL1/ABL1 ratio <0.01% by PCR) and major molecular response (MMR; BCR-ABL1/ABL1 ratio <0.1% by PCR).

Results: Among 46 patients with Ph+ ALL, 74% (n=34) were in imatinib group and 17% (n=8) in dasatinib group. 9% were treated with other or no TKI (1 ponatinib and 3 with no TKI). Thirty-eight percent (n=13) of patients in imatinib group and 13% (n=1) in dasatinib group switched to a different TKI due to adverse effects or failure to achieve remission. There was a trend towards increased 2-year LFS for patients on dasatinib (HR 0.40, 95% CI: 0.14-1.14, p=0.09) and no difference in 2-year mortality (HR 1.00 95%CI: 0.46-2.17, p=0.99). 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).

Summary/Conclusions: The results of this study suggest that dasatinib is superior to imatinib in Ph+ adult ALL, resulting in improved 2-year LFS and OS. Dasatinib is a suitable first-line agent with similar OS and better LFS compared to imatinib.
Results: Eight of 21 (38%) patients exhibited an isolated t(4;11) translocation. Additional chromosome abnormalities (ACAs) were revealed in 11 (52%) patients, including 8 (42%) subjects with 3 and more chromosome aberrations. In univariate analysis, significance was shown for clinical stage at HSCT (1st remission vs other stages, 75% vs 0%; p<0.001 for OS; 58% vs 0%, p<0.001 for EFS), complex chromosomal aberrations (<3 abnormalities vs ≥3 abnormalities, p=0.04 for OS; 46% vs 0%, p=0.04 for EFS). According to multivariate analysis, the clinical stage at HSCT (HR 26.6, 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 outcomes.

Summary/Conclusions: The study has shown the stage of disease at the moment of allo-HSCT to be an independent prognostic factor in a mixed cohort of KMT2A- AFI/ ALL patients treated with HSCT. The good results of allo-HSCT can be obtained using a haploidentical transplantation from parents that removes the problem of searching the HLA-matched donors in the Registers and, therefore, greatly simplifies the treatment.

PB1646
DERMATOLOGIC COMPLICATIONS ASSOCIATED WITH TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF ACUTE LEUKEMIA
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Background: Despite of targeted effects of tyrosine kinase inhibitors (TKIs), they are not absolutely selective in relation to their target. Hair pigmentation is regulated by factors including the interaction of the ligand stem cell factor (SCF) with its class III receptor tyrosine kinase, c-kit. Hair depigmentation observed during therapy TKI with action directed against class III receptor tyrosine kinase (PDGFRα, PDGFRβ, C-KIT, CSF1R, FLT3). But other TKI such as BCR/Ab1

Aims: To describe the spectrum of skin and hair reactions in patients with acute leukemias (Ph+/Ph- acute lymphoblastic leukemia and acute myeloid leukemia) during the treatment by second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor (sorafenib).

Methods: From 2016 to March 2017 6 patients (pts), age 24-53 (median 29,5), 1 male, 5 female, received second or third line therapy with target tyro-
sine kinase inhibitors in National Research center for Hematology. One pt (pt 1) with AML had been receiving chemotherapy (decitabine, cytarabine, idarubicin) with continuous treatment of sorafenib. Three pts with Ph+/ All received TKIs. Two of them with T315I mutation (pts 2, 3) received ponatinib and one pt (pt 4) without molecular remission on dasatinib and nilotinib ther-
apy, received second-generation TKI (bosutinib). One pt with B-ALL was treated by sorafenib due to refractory disease on the first-line therapy (pt 5). And one patient (pt 6) with T-cell ALL received sorafenib with nelarabine con-
taining chemotherapy due to early relapse after allogenetic stem cell trans-
plantation.

Figure 1.

Results: All of the 6 patients who had taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib developed dermatologic reactions (skin rash or grey hair). Generalized maculo-papular skin rash grade II evolved after two weeks of sorafenib treatment in pt1. Both patients on ponatinib therapy developed localized maculo-papular skin rash grade I in pt 2 after 8 weeks of therapy. In pt 3 after 6 weeks of ponatinib treat-
ment gray hair observed. Skin rash with pigmentation grade I evolved in pt 3 after 12 weeks of therapy. Pt 4 had gray hair after 12 weeks second-generation TKI (bosutinib) treatment. Palm-plantar erythrodysaesthesia syndrome grade II and hair and total skin depigmentation were evolved after 2 weeks and after 4,5 months respectively observed during the sorafenib treatment. Pt 5 (pt with
psoriasis anamnesis) Pt 6 developed localized maculo-papular skin rash grade I after 5 weeks of sorafenib treatment. Despite of all patients developed der-
matological side effects, temporarily discontinuation of TKI therapy was required in only three (50%) cases. In the other cases the treatment was con-
tinued. The therapy was restarted in all pts with temporarily discontinuation after skin lesions disappearing (Figure 1).

Summary/Conclusions: Dermatological adverse events in acute leukemia pts who have taken second-generation TKI (bosutinib), third-generation TKI (ponatinib) and multikinase inhibitor sorafenib they were not serious. Temporar-
yly dose reduction or interrupting of TKI therapy led to complete remission of tkI therapy led to complete remission of skin lesions. Restarting TKI at full dose did not lead to dermatological adverse reactions reappearance. Moreover, the temporary cancellation did not reduce its effectiveness.

PB1647
CYTOKINE RELEASE SYNDROME AFTER THE FIRST INTRATHECAL CHEMOTHERAPY IN A PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH AN EARLY MENINGEAL RELAPSE
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Background: Central nervous system (CNS) is a frequent site of recurrence in childhood acute lymphoblastic leukemia (ALL), and Triple Intrathecal Therapy (TIT) with Methotrexate (MTX), Cytarabine (ARA-C) and hydrocortisone, at the moment of the mainstay of treatment of CNS relapse. Severe neurotoxicity is well known TIT complication, usually related to repeated infusions and neurotoxic compitant systemic drugs.

Aims: We describe a case of a massive acute leukoencephalopathy after only one TIT, in a 5 year-old child with an early isolated CNS relapse of ALL (26 months after the first diagnosis), rapidly proceeding to comatose status.

Methods: At admission for disease restaging at the end of first-line trial, the child showed physical and neurological examination completely negative, such as haematological, bioumoral and ultrasound findings. The cerebrospinal fluid (CSF) appeared turbid and liquoral pressure increased. CSF count showed 8100 cells/μl: morphology and flow citometry confirmed an early isolated CNS relapse. Due to the abnormal pleocytosis, TIT administration associated with oral dexamethasone was suddenly performed without any other concomitant chemotherapy. To prevent acute toxicities from tumor lysis syndrome, the patient received hydration, allopurinol, acetazolamide and prophylaxis of seizures with levetiracetam. After few hours from TIT, the child developed severe headache followed by skin urticarial rash, high blood pressure and hallucinations, rapidly evolving in flacid paralysis of lower extremities. A brain resonance (MRI) showed diffuse areas of hyperintensity of white matter, par-
timents of subcortical and subcerebellar areas, cerebellar region, optic chiasm and brainstem in T2-Flair sequences; spinal cord showed edema, espe-
cially in lombar region. The MRI pattern was interpreted as diffuse grade IV leukoencephalopathy of probable toxic nature. The child, 30 h after TIT, was transferred to intensive care unit for progressive ascending paralysis and respiratory distress that required intubation. During the following days, other three diagnostic lumbar puncture were performed that showed significant reduction of blasts cells (20, 10 and 0 cells /μl, respectively).

Results: Patient persisted in deep coma for 5 days, until he restart a sponta-
neous breathing. After waking up, the child showed rapid neurological amelio-
ation, seizures reappearance, exophses, spontaneous movements in hands, hand and feet fingers. The subsequent MRI highlighted improvement of hyper-
intensity at midbrain, brainstem and bridge brain areas and spinal cord with persistence of altered signals in subcortical white matter. The visual evoked potentials were normal and the motor and sensory conduction velocity 400 appeared slowed without axonal damage; EEG showed slow waves spread. At the moment, after three week from severe neurological complication, the child is fully awake, moving all four limbs, but requires motor and phoniatric rehabilitation. Systemic chemotherapy with high-dose MTX and IT ARA-C is restarted without any additional neurotoxicity. Dosage of CSF levels of inter-
leukin 6 and its soluble receptor is ongoing.

Summary/Conclusions: Although leukoencephalopathy following IT MTX or ARA-C administration are described, the severity and rapidity of event’s onset, associated with CSF remission after a single TIT administration, suggests us that neurotoxicity could be related to massive blast cytolyis with subsequent release of these syndromes and an inflammatory machinery. This syndrome is a frequent complication of blinatumomab or chimeric antigen receptor T-cells administrations. The CSF IL-6 dosing could clarify the patho-
genesis of the event.
SEVERE HYPOFIBRINOGENEMIA ASSOCIATED WITH IMATINIB AND PREDNISONE THERAPY IN PHILADELPHIA CHROMOSOME–POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Hypofibrinogenemia associated to acute lymphoblastic leukemia (ALL) is rare and usually due to L-asparaginase. Consumption coagulopathy or therapy-related hematotoxicity are other possible explanations. Severe hypofibrinogenemia, not linked to the causes listed, was rarely reported and a role of steroid therapy on fibrinogen metabolism was suggested.

Aims: Our aim was to identify the incidence of severe hypofibrinogenemia during induction phase in a cohort of consecutive ALL patients and to assess its impact on clinical decision-making.

Methods: In order to avoid confounding factor due to L-asparaginase, we revisited 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 6x10^9/L). Coagulation tests were normal (fibrinogen median level 380 mg/dl). Severe hypofibrinogenemia developed between 6 and 15 days after beginning treatment and lasted between 4 and 48 days. Fibrinogen nadir ranged from 47 to 100 mg/dl (median 61 mg/dL); reduced plasma fibrinogen levels at functional tests were also confirmed to immunological assays. During fibrinogen nadir, D-dimer was positive in all patients, but stable compared to the outset. Antithrombin, coagulation factors, activated partial thromboplastin and prothrombin time, common liver function tests remained in a normal range; platelet counts showed a trend to normalization. Early clearance of peripheral blood blasts was observed and when hypofibrinogenemia appeared no blast cells were detectable. At the end of induction bone-marrow evaluation demonstrated the absence of BCR-ABL transcript by qualitative RT-PCR. There were no bleeding events and only one patient received a prophylactic transfusion of fresh-frozen plasma (10 ml/kg) for fibrinogen <50 mg/dl on two occasions. Normal fibrinogen levels (≥165 mg/dl) were recovered at the end of steroid therapy.

Summary/Conclusions: We observed severe hypofibrinogenemia in Ph+ALL patients older than 65 years treated with imatinib and high-doses steroid, while normal fibrinogen levels were detected in younger Ph+ALL during intensive chemotherapy plus imatinib. In our experience, hypofibrinogenemia was not associated to major bleeding events, although its clinical significance should be investigated in larger series. Fibrinogen may recognize multiple metabolic pathways, also unrelated to in vivo coagulation and fibrolysis; 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.
Acute myeloid leukemia - Biology

PB1650

MUTATIONAL ANALYSIS OF 231 DE NOVO AML PATIENTS BELOW 60 YEARS WITH CURATIVE THERAPY

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Background: Acute myeloid leukemia (AML) is an aggressive cancer disease of the myeloid lineage of blood cells, characterized by rapid growth of undifferentiated myeloid precursors. Analysis of the spectrum of somatic mutations in leukemic cells may help to improve the identification of individual prognostic subgroups of patients as well as to observe clonal evolution in the course of AML treatment.

Aims: The aim of the project is to identify somatic alterations in genes related to AML using next generation sequencing (NGS) in large cohort of AML patients from Czech Republic and to determine their frequency and mutual coexistence.

Methods: The analyzed group consists of 231 de novo consecutively diagnosed AML patients with curative therapy below 60 years from five hematological centers. The NGS libraries are prepared from peripheral blood samples from diagnosis using ClearSeq AML panel (Agilent Technologies) and sequenced on MiSeq and NextSeq machines (Illumina). As positive are determined mutations with variant allele frequency (VAF) at least 2%.

Results: At least one somatic mutation (median 2; range 0-6) was identified in 204 (88.3%) patients with de novo AML. In total, 526 recurrent mutations in 19 genes were identified. The most frequently mutated genes were: FLT3 91/231 (39.4%; from this FL73-TID 69/231 [29.9%] and FLT3-TKD 22/231 [9.5%]), NPM1 90/231 (39.0%; mutation type A 71/90 [78.9%], type B 11/90 [11.1%], other types 10/90 [10.0%]), DNMT3A 68/231 (29.4%; mutations in codon R882 49/68 [72.1%]), NRAS 51/231 (22.0%; the most frequent mutation G12D 17/51 [22.0%]; 1151 patients [21.6%] contain more than one mutation in NRAS gene), IDH2 35/231 (15.2%) and 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 regulating cells and interrogated gene expression data with different bioinformatic tools. 3. AML mouse xenograft model was used to examine the in vivo function of LIN28B.

Results: We first showed that increased LIN28B expression was associated with worse survival in AML patients. We demonstrated that targeting LIN28B in AML cells resulted in cell cycle arrest, inhibition of cell proliferation and colony formation, which was induced by de-repression of let-7 miRNA. On the other hand, overexpression of LIN28B promoted cell proliferation. Mechanistic studies revealed that inhibition of LIN28B induces metabolic changes in AML cells. IGF2BP1 was confirmed to be a novel downstream target of LIN28B via let-7 miRNA. 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 leuke-mogenesis and provide a rationale to target this pathway as effective therapeu- tic strategy.

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 et al. Leukemia 2006). Patients were considered positive when presented >1 NPM1 sample positive or/and one sample NPM1 >0.02%. Cox regression was used for univariate analysis.

Results: Patients were segregated in 2 groups: Relapse patients (Group 1: 32.2%, 11/34) and no relapse patients (Group 2: 67.6%, 23/34). Group 1 presented MRD NPM1 positive in 9/11 (82%) of patients, the time from NPM1 to relapse was 4.6 months (1.6-24), NPM1 mean was 1.7 (0.03-9). Group 2 presented MRD NPM1 negative (<0.02% y/ or 1 determination) in 21/23 (91%) patients. Univariate analysis was performed and our results show that age, leukocyte, LDH and MRD NPM1 are prognostic factors for cumulative incidence of relapse (Figure 1).
Summary/Conclusions: NPM1 is a useful marker for MRD quantification in AML patients undergoing intensive therapy. NPM1+ positive during follow-up is associated with a higher probability of relapse.

PB1654
AT101 ELIMINATES AML STEM CELLS VIA ACTIVATION OF INTRINSIC APOPTOTIC PATHWAY AND PARTICIPATION IN DNA DAMAGE RESPONSE

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Background: Leukemia stem cells (LSCs) are considered as the main reason for treatment failure and relapse in acute myeloid leukemia. Overexpression of Bcl-2 anti-apoptotic proteins is associated with the survival and self-renewal of LSCs.

Aims: To observe the effect for AT101 to eliminate AML stem cells and its underlying mechanism.

Methods: Use CD34+/CD38-/CD123+ KG1a 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+ KG1a 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 potentiality 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 part 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, irudabcin and cytarabine) to treat CD34+CD38-/CD123+ KG1a cells and primary refractory or relapsed AML CD34+ cells.

Results: Here, we reported 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, irudabcin and cytarabine) in CD34+CD38-/CD123+ KG1a cells and primary refractory or relapsed AML CD34+ cells, reflected by inhibition of cell proliferation and induction of apoptosis in vitro. Mechanistically, these effects 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+ KG1a 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: A new strategy to eliminate LSCs in AML is urgently needed.

Methods: We collected data of all the APL referred to our institution from 2014. Within 23 patients, we encountered 20 new diagnosis and 2 relapse of APL. We analyzed blasts in samples obtained from Bone Marrow with Single Nucleotide Polymorphisms Array Cytoscan HD.

Results: We compared copy number alterations in both relapsed patients with alterations detected in the pool of 20 newly diagnosed APL and we found specific signatures of CNVs for each patient. There were several copy number alterations related to each patient: the first patient presented gain of ROBO2, GRIP1, CTNNB1, SOX6, PBX1, GRIK2, CDKAL1 and loss FAP1, CREBBP, SBF1; the second patient presented gain of ROBO1, MAPK10, CADPS2, APBA1 and loss of GRIP1 and MYB. Subsequently we focused our attention on ROBO and GRIP1 genes because they were altered in both relapsed patients: ROBO proteins are associated to K channels while GRIP1 is involved in various critical functions, for example in androgen receptor binding, beta-catenin binding, glucocorticoid receptor binding, and it is also a regulator of glutamate metabolism, a well-known pathway in Leukemic Stem Cells.

Summary/Conclusions: By the analysis of ROBO 1-2 and GRIP1 at the 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-cel specific moloney murine leukemia virus integration site-1 (BMI-1) genes are stem cell genes that modulate stem cell pluripotency and may play a role in leukemogenesis. Leukemic stem cells (LSCs) have been implicated in being the origin of the leukemic blast, therapy resistance and recurrence.

Aims: The current study aimed at characterizing the expression pattern of SALL4 and BMI-1 genes in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), in patients who have achieved complete remission (CR), and in CML disease progression.

Methods: Real-time polymerase chain reaction was used to assess the gene expression patterns in 106 myeloid leukemia patients: 54 de novo AML (43 at time of diagnosis, 11 in CR), and 52 CML (31 in chronic phase (CP), 11 in deep molecular response (MR) & 10 in accelerated/blastic phase (AP/BP), and in 21 non malignant bone marrow samples.

Results: SALL4 gene expression was increased in AML patients, AML-CR, & CML-CP (median= 5.180, 4.604 & 14.125 respectively). No significant difference was observed between de novo AML and AML in CR patients. CML-CP patients showed a significantly higher percentage of patients with a high SALL4 expression as compared to both CML-MR & CML-AP/BP (p=0.033). BMI-1 gene expression was not found to be increased in any of the patient groups.

Summary/Conclusions: Our data describe altered SALL4 gene expression in different phases of myeloid leukemia. The role of BMI-1 gene needs further delineation to determine its significance.
AN INVESTIGATION INTO THE ROLE OF S100A8 AND S100A9 IN ACUTE MYELOID LEUKAEMIA
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Background: Acute myeloid leukemia (AML) is the a haematological malignancy characterised by the over proliferation and block in differentiation of clonally transformed leukaemia potential progenitors such as S100A8 could assess the progression and remission of AML.

Aims: S100A8 and S100A9 (Ca2+ binding helix E-loop-helix-F hand), are inflammatory markers which are also suggested to promote chemoresistance by stimulation of autophagy. Microarray data from the Chevassut lab shows that both S100A8 and S100A9 transcripts are downregulated by the BET-bromodomain inhibitor JQ1 in AML cell lines. We aimed to investigate this response in AML patient bone marrow samples and cell lines.

Methods: We used AML cell line including OCI-AML2, OCI-AML3 and THP-1 in addition to AML patient bone marrow samples and healthy volunteer sample. We carried out RT-qPCR and immunocytochemistry and western blotting techniques to look at levels of S100A8 and S100A9 in samples.

Results: Here we show that levels of S100A8 and S100A9 miRNA levels are suppressed in response to JQ1 in the AML cells lines OCI-AML2, OCI-AML3 and THP-1. We find also that protein levels of S100A8 and S100A9 are downregulated in response to JQ1 in OCI-AML3. In bone marrow samples of 17 AML patients with different cytogenetic profiles, the relative expression of S100A8 and S100A9 was found to be variable amongst the samples but also in comparison to OCI-AML3 cell line. In further experiments using AML patient bone marrow samples, treatment with JQ1 showed suppression of S100A8 and S100A9 in some patient samples but enhanced expression in other bone marrows. In peripheral blood samples of healthy volunteers, we found that treatment with JQ1 showed notable suppression of both S100A8 and S100A9 with a greater suppression being observed in the 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 line 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.

PB1660 SUCCESSFUL COVERAGE OF DIFFICULT TO SEQUENCE GENES (CALR, CEBPA, and FLT3) ASSOCIATED WITH MYELOID DISORDERS USING A HYBRIDISATION-BASED ENRICHMENT APPROACH PRIOR TO NEXT-GENERATION SEQUENCING
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Background: The application of short read NGS for research into myeloid disorders such as myeloproliferative neoplasms (MPNs) and acute myeloid leukemia (AML) faces some challenges including a high error rate and the inability of some genes to be covered with current NGS read lengths and the high error rate associated with these samples. For further work, more detailed insight into the mechanisms of action and potential use of genes of interest is required.

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 projects for each of these difficult to sequence genes. The results clearly show that this approach can reliably detect and accurately size (including low allele frequency) insertions and deletions of up to 52 bp in CALR (exon 9), SNVs and deletions in CEBPA with a de-duplicated depth in excess of 2000x as well as ITD's between 24 and 201 bp in FLT3.

Summary/Conclusions: This approach is suitable for the analysis by NGS of these difficult genes and therefore removes the requirements for supplementary approaches to analyse these difficult genes, such as Sanger sequencing (CEBPA) and fragment analysis (CALR and FLT3).

PB1661 ASSOCIATION OF miRNA EXPRESSION PROFILES WITH FUNCTIONAL AND MOLECULAR ACUTE MYELOID LEUKAEMIA CATEGORIES
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Background: Development of high-throughput technologies such as Next Generation Sequencing (NGS) allowed the identification of recurrent mutated genes in Acute Myeloid Leukemia (AML), and new molecular markers which help refine patients’ classification in different risk groups.

Epigenetic alterations such as aberrantly expressed microRNAs (miRNAs) also play a prominent role on the pathogenesis of AML, participating in processes such as cell development, differentiation, proliferation and apoptosis. Therefore, aberrant miRNA expression can affect signaling and metabolic pathways, directing cancer cell biological behavior.

Recently, several studies have classified AML according to different criteria. The classification proposed by the TCGA and Papaemmanuil et al. Associations of miRNA expression profiles with altered categories could help understand the molecular mechanisms that lead to leukemogenesis.

Methods: CD34+ cord blood progenitor cells from 5 healthy donors and 7 CD34+ NK-AML samples with >70% blasts were obtained. Total RNA from each of these samples was hybridized onto an Array miRNA 3.0 chip (Affymetrix) in order to identify deregulated miRNAs. The most deregulated miRNAs were validated by qRT-PCR (miScript) in an independent cohort of 73 patients. Muta
tional analysis was performed by Next Generation Sequencing using the AML Community Panel with the Ion Torrent System (Life Technologies).

Results: We found a profile of 6 miRNAs upregulated and 61 miRNAs downregulated in NK-AML vs CD34+ cells. Validation by qRT-PCR confirmed miR-494 (p=0.028) and miR-499 (p=0.035) were up-regulated and miR-27b (p=0.022), miR-95a (p=0.001), miR-146b (p=0.031), miR-15b (p=0.006) and miR20b (p=0.001) were down-regulated in NK-AML. Interestingly, some of the deregulated miRNAs were significantly associated to a functional category according to the TCGA classification. Therefore miR-146b was down-regulated in AML with mutations in myeloid transcription factors (p=0.025). Low expression of some of these RNA caused the activation of the JAK2/STAT5 signaling pathway, which increases transcription. miR-4668 was down-regulated in AML with mutations in activation pathways genes (p=0.004), several target predictors propose RASG6F1A and BRAF as targets of this miRNA. Thus, under-expression of this miRNA could cooperate with mutations leading to the activation of signaling pathways. Regarding to Papaemmanuil’s molecular classification, miR-494 was up-regulated in IDH2-R172Q category (p=0.009). High levels of this miRNA are associated with lower expression of TET, specially TET1. Therefore, high levels of miR-494 could contribute to the hypermethylation signature of IDH (Acute Myeloid Leukaemia) subtype.

Summary/Conclusions: 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.

PB1662 PROTEOMIC APPROACH TO IDENTIFY MOLECULAR TARGETS OF HALOFUGINONE IN ACUTE MYELOID LEUKEMIA
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Background: Halofuginone (HF) is a halogenated derivative of Febrifugine, which is a molecule isolated from the plant Dichroa febrifuga. It has been demonstrated that Halofuginone exhibits anti-fibrotic, anti-cancerogenic, anti-inflammatory and pro-angiogenic effects. Previously, we have reported that treatment of AML with HF has anti-leukemic properties in vitro and in vivo in acute promyelocytic leukemia (APL), reducing tumor growth through the induction of apoptosis and by stimulating the synthesis of the TGF-β protein and activating its downstream targets. In addition, HF presented anti-angiogenic effects by modulating the level of pro and anti-angiogenic factors including VEGF. However, it is unknown whether HF has anti-leukemic properties in other subtypes of acute myeloid leukemia (AML) and HF targets were not determined yet.

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®.

Background: Halofuginone (HF) is a halogenated derivative of Febrifugine, which is a molecule isolated from the plant Dichroa febrifuga. It has been demonstrated that Halofuginone exhibits anti-fibrotic, anti-cancerogenic, anti-inflammatory and pro-angiogenic effects. Previously, we have reported that treatment of AML with HF has anti-leukemic properties in vitro and in vivo in acute promyelocytic leukemia (APL), reducing tumor growth through the induction of apoptosis and by stimulating the synthesis of the TGF-β protein and activating its downstream targets. In addition, HF presented anti-angiogenic effects by modulating the level of pro and anti-angiogenic factors including VEGF. However, it is unknown whether HF has anti-leukemic properties in other 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, MV4-11, 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 – Human-Phospho-Kinase Array to verify the possible tyrosine kinases and signaling pathways that could be modulated by HF. To analyze the in-vitro effect of HF, 1x10^6 Kasumi-1 and THP-1 cells were treated with vehicle or HF (100 ng/ml). Cell viability was assessed using the Alamar Blue assay. The primary targets of HF in both Kasumi-1 and THP-1 cells, HDACs, were analyzed using a HumanHDAC array. We used a HumanPhospho-Kinase array to confirm the possible signal transduction pathways modulated by HF.

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 HF-1 to HF, we did not detect significant differences 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 downregulated only in THP-1, while the levels of STAT3 S727 and STAT5a/b were significantly decreased by HF treatment only in Kasumi-1 cells. This comparative analysis suggests that the sensitivity to HF may be dependent on inhibition of STAT3/5 pathway.

Summary/Conclusions: In summary, our results suggest that HF may be effective against AML cell lines through the blocking of the JNK cascade and down-regulated only in THP-1, while the levels of STAT3 S727 and STAT5a/b were significantly decreased by HF treatment only in Kasumi-1 cells. This comparative analysis suggests that the sensitivity to HF may be dependent on inhibition of STAT3/5 pathway.
existing hypomethylating-based protocols: a) high gene specificity b) lower cytotoxicity and c) absence of drug based off-target side-effects. In the short term, this research can lead to the identification of novel key regulators of leukemogenesis and new targets for therapeutic treatments; in the long term pave the way for development of RNA-based gene demethylating agents for cancer treatment.

PB1665

JQ1 AND CURCUMIN COMBINED TREATMENT SHOWS SYNERGIC EFFECTS IN MLL-REARRANGED LEUKEMIA CELL LINES

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Background: MLL-rearranged leukemia accounts for ~70% of infant and ~10% adult acute leukemias, featuring a particularly poor prognosis and high risk of relapse. Our main field of study is AML, in which nearly 50% of total cases accounts for t(9;11) translocation, the remaining 50% predominantly includes t(6;11)(q27;q23), t(10;11)(p12;q23), t(11;19)(q23;p13.1) and t(11;19)(q23;p13.3). A 2% of AML total cases, however, is characterized by t(4;11) translocation, which is a marker of bad prognosis and it’s, so far, poorly characterized. A key feature of MLL-rearranged leukemia is cMyc overexpression, a well-known oncogene involved in several types of cancer. JQ1 is a novel molecule, which prevents cMyc expression binding an important bromodomain protein, BRD4. Moreover, Curcumin, a natural compound, inhibits HATs enzymes preventing lysine 14 acetylation on histone H3 (AcH3K14), a 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 therapeutic alternative to standard chemotherapy and to deeply investigate features underlying the molecular pathogenesis in pediatric MLL-rearranged pediatric AML.

Methods: Four human leukemia cell lines with MLL fusion protein have been employed in this study. RS4-11, MV4-11 projecting MLL-AF4 and THP1, MOLM13 expressing MLL-AF9 fusion genes. 5μM and 10μM Curcumin were employed in this study: RS4:11, MV4:11 expressing MLL-AF4 and THP1, 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 synergic significiant 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 expression targets of the drugs (cMyc, AHC3K14). Finally, in MLL-AF4 cell lines, curcumin and JQ1 together induced a significant decrease in mir-99a expression.

Summary/Conclusions: We 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 AHC3K14 expression, confirming that both curcumin and JQ1 have a synergic effect. Additionally, we revealed a significant reduced expression of mir-99a, a well know oncomiR reported to act as negative regulator of differentiation and involved in drug-resistance, typically up-regulated in pediatric AML and ALL.

Figure 1.

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 expression targets of the drugs (cMyc, AHC3K14). Finally, in MLL-AF4 cell lines, curcumin and JQ1 together induced a significant decrease in mir-99a expression.

Summary/Conclusions: We 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 AHC3K14 expression, confirming that both curcumin and JQ1 have a synergic effect. Additionally, we revealed a significant reduced expression of mir-99a, a well know oncomiR reported to act as negative regulator of differentiation and involved in drug-resistance, typically up-regulated in pediatric AML and ALL.

PB1666

TP33B AND 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, p53ß and p53y, without oligomerization domain (stop codon is localized in exon 9b). These isoforms can be present in acute myeloid leukemia (AML) cells. p53ß binds to BAX promoter and can induce apoptosis independent from p53 wt. p53ß has influence on activation of CEBPA which is associated with cell cycle regulation, especially cell cycle arrest and plays also role in cell differentiation. Generally, it is a transcription factor expressed during myeloid lineage development, from progenitor cells to mature granulocytes. Various mutations of CEBPA gene are described. Among them N-terminal and C-terminal mutations, mostly insertions and deletions, are often present.

Aims: The goal of the study was to assess mutational status of NPM1, CEBPA and FLT3 in association with TP53beta and TP53gamma expression levels.

Methods: 75 NK AML patients were included in the study. NPM1, CEBPA and FLT3 gene mutations were analyzed by direct sequencing. TP53ß and TP53y expression levels were assessed in 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, TP53ß and TP53y 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 TP53ß was much higher (ΔΔCt 43,11) than TP53y (ΔΔCt 10,85; p<0,05). Furthermore, expression level of TP53y 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 TP53ß or TP53y 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 expression. 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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Aims: We would like to explore a potential synergic effect between JQ1 and Curcumin molecules in the attempt to develop a novel therapeutic alternative to standard chemotherapy and to deeply investigate features underlying the molecular pathogenesis in pediatric MLL-rearranged pediatric AML.
Background: Whole-genome sequencing has revealed acute myeloid leukemia (AML) as a very complex and dynamic disease. Epigenetic modulation is among the functional categories of the mutational landscape in AML. According to recent reports, suppression of the epigenetic reader BRD4 with small-molecule inhibitors (BET-i) results in antigenic reprogramming. 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 not associated with a better response. It has been recently suggested that antileukemic effect of BET-i could be due to c-myc suppression and that high Bcl-2 levels may target those patients that would benefit of BET-i. We believe that establishing the expression profile of epigenetic modifiers in AML may help in the identification of patients that could benefit from BET-i.

Aims: We wanted to get a better insight regarding the expression profile of epigenetic modulator in AML of intermediate risk by studying: 1) expression levels of EZH2, ASXL1, BRD4, c-myc and 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 a control gene.

Results: mRNA expression levels were positively associated with EZH2 (Spearman's ρ = 0.285, p < 0.021) and BRD4 with-c-myc (Spearman's ρ = 0.420, p < 0.001), Bcl2 (Spearman's ρ = 0.471, p < 0.001) EZH2 (Spearman's ρ = 0.4655, p < 0.008) and ASXL1 (Spearman's ρ = 0.949, 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% CI 15.1-38.9) compared to those with low expression (median OS 12 months, 95% CI 6.4-23.7), although the association was not statistically significant (p = 0.196) probably due to the limited series size. Protein levels of Bcl2 and c-myc correlated with those of mRNA, but not for BRD4, although other antibodies should be tested in order to confirm these results. CHIP analysis in HL60 cell lines confirmed the binding of BRD4 to c-myc promoter.

Summary/Conclusions: The positive association observed between EZH2 and ASXL1 agrees with the fact that both cooperate in the epigenetic repressive complex PRC2. The binding of BRD4 to the c-myc enhancer region 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 BET-i.

PB1668

FLOW CYTOMETRY IMMUNOPHENOTYPING IN CEBPA-DM DE NOVO AML: BIOLOGIC AND PROGNOSTIC RELEVANCE.
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Background: CEBPA is a transcriptional co-factor of RUNX1 which play a major role in the fate decisions associated with physiologic myelopoiesis. Biallelic CEBPA mutations (dcm) 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 dcm AML.

Methods: Twenty-five patients with de novo CEBPA dcm AML who where enrolled on the AML-03 and AML-12 protocols of the Spanish CETILAM cooperative group were included in this study. The immunophenotypic analysis was performed on erythrocyte-lysed bone marrow (BM) samples obtained at diagnosis. Antigenic expression of leukemic cells was systematically analyzed by multiparametric flow cytometry using four-color staining. The antigens studied were: CD45, CD34, HLA-DR, CD10, CD20, CD19, CD2, CD33, CD7, CD117, CD66, CD13, CD64, CD36, CD56, CD14, CD123, CD61, CD42b, glycophorin, CD71, CD11b, myeloperoxidase, CD79a, Td, lysozyme and lactoferrin. At least 10,000 events/tube were measured. Analytical gates were established according to CD45 reactivity and to FSC/SSC pattern. Positivity threshold was established at 20%. The FACS-DIVA,Paint-a-Gate and Infinicyt software programs were employed for analysis. Amplification of overlapping PCR products covering the whole CEBPA coding sequence followed by Sanger sequencing were used to investigate CEBPA mutations. FLT3-ITD, NPM1, MLL-PTD, WT1 and GATA2 mutations were also investigated by conventional PCR-based molecular methods.

Results: Antigen reactivity was as follows: CD45 (39/39, 100%), CD15 (35/39, 90%), CD34 (36/39, 92%), HLA-DR (39/39, 100%), CD33 (39/39, 100%), CD2 (2/39, 5%), CD7 (36/39, 92%), CD117 (39/39, 100%), CD13/37/39, 95%), CD56 (6/39, 15%), CD36 (6/39, 15%), CD123/39, (100%), CD14 (1/39, 0.02%), CD71 (38/39, 97%), myeloperoxidase (38/39, 97%). In nine cases CD36 and/or CD56 expression on leukemic blasts was greater than 20% Those CD36/CD56+ cases had a shorter overall survival and leukemia free survival (see graph). Four out five tested CD36/CD56+ cases also showed GATA 2 mutations. An additional CD36/CD56+ case had a FLT3-ITD. In three out 39 cases (7%) a population showing cytoplasmic CD79a reactivity was detected (8%, 11%, 14% of the neoplastic population, respectively). Two of those cases had also a FLT3-ITD.

Summary/Conclusions: CEBPA dcm cases showed an homogeneous immunophenotype with positivity for CD45, CD7, CD34, CD123, CD117, HLA-DR, CD71, CD33, CD13 and CD15. CD36 and/or CD56 overexpression was detected in a subgroups 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 dcm AML cases. Small leukemic populations with B-cell markers are not uncommon in CEBPA dcm 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 taken after induction therapy, reflecting changes in protein expression due to treatment. Comparing the proteinomes, we found 11 spots that differed significantly (fold change of +/- 1.5 and p < 0.05). Of these, seven proteins were up-regulated and four were down-regulated at time of diagnosis (before treatment) compared to after induction treatment. Nine of these spots correspond to low molecular weight proteins (<40 kDa) and 2 spots have a molecular weight between 40-60 kDa. Based on the molecular weight and isoelectric point information of these spots we were able to search for proteins reportedly involved in leukemia, in order to propose possible identities (see Table 1). In terms of biological processes, four proteins (eIF3A, HSP27, 14-3-3 protein zeta/delta, and GST-P) are involved in the regulation of apoptosis. The F-actin-capping protein subunit beta could also be of interest, as reorganization of F-actin reflects unique characteristics of the differentiation process in promyelocytic leukemia cells. RuvB-like 2 is a positive regulator of histone acetylation and DNA repair. GRBP2 is a protein involved in the MAFK cascade and regulation of PI3K signaling, pathways regulating diverse cellular functions altered in leukemogenesis such as proliferation, differentiation, and apoptosis. Alpha-encephalins is a key glycolytic enzyme; however, it has been shown to be a multifunctional protein involved in cancer. It promotes cell proliferation by also regulating the MAPK and PI3K pathways. Transaldolase is part of the pentose-phosphate pathway. Annexin II acts in angiogenesis and has multifaceted role in human health and disease.

Summary/Conclusions: The protein expression profile of AML patients changes after induction treatment. We found 11 spots that differed significantly, and propose possible identities for these. Further analyses are pending in order to experimentally establish the identities and correlate with response to treatment.

PB1670
AMP-ACTIVATED PROTEIN KINASE ACTIVITY INTERFERS WITH OVEREXPRESSION OF NUCLEOPHOSMIN IN CYTARABINE-INDUCED CHEMORESISTANT AML CELLS
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Background: Cytarabine is a chemotherapeutic drug used alone or in combination with other anticancer drugs to treat acute myeloid leukemia (AML). New treatment strategies are emerging to enhance the anti-cancer effect and decrease the toxicities. Nucleophosmin (NPM1 or B23) is a ribosomal protein localized mainly in nucleolus, and multifunctional enzyme in cancer cell growth and proteinsynthesis. AMP-activated protein kinase (AMPK) is a critical energy sensor to regulate homeostasis and plays a potential role for anti-cell proliferating activity.

Aims: We investigated the effects of AMPK activation on the cell death (apoptosis), cell cycle distribution and multifunctional enzyme in cancer cell growth and proteinsynthesis. AMP-activated protein kinase (AMPK) is a critical energy sensor to regulate homeostasis and plays a potential role for anti-cell proliferating activity.

Methods: We used the HL-60 (FAB M2) cells were exposed to the different drug combinations including cytarabine and AMPK activators. The molecular mechanisms of AMPK activation in HL-60 cells treated with or without cytarabine, an anti-leukemic drug, to predict the mechanisms responsible for AML cells chemoresistance.

Results: We found that cell apoptosis (36.27 - 42.11%) showed little dependence on cytarabine concentrations (10, 100, and 1000 mM), while the overexpression of NPM1 increased proportionally with drug dependence, indicating the drug-induced cell resistance. In the same point, cytarabine also inhibited the phosphor-activity (Thr172) and expression level of AMPK, which has mTOR-p70S6K pathway-repressor activity. As expected, single cytarabine treatment increased the ratio of p-mTOR/mTOR and p-p70S6K/p70S6K. Co-treatment of AMPK activator (phenformin or AICAR) in cytarabine-resistant HL-60 AML cells inhibited significantly the induction of NPM1 overexpression level with the decrease of phosphor-activities of mTOR and its substrate p70S6K, resulted in the accelerated cell apoptosis.

Background: Leukemia cells are limitless cell sources for initiation and maintenance of leukemia. Telomere-binding proteins are key regulators for various diseases, including leukemia. Therefore, targeting telomere-binding proteins is considered as a promising therapeutic strategy for treatment of leukemia.

Aims: We aimed to explore whether quercetin, a natural flavonoids, could regulate telomere-binding proteins expression to inhibit proliferation and induce apoptosis in acute myeloid leukemia(AML) THP-1 cells.

Methods: 1. In vitro: (1) We cultured human AML THP-1 cells. (2) The cells were treated with different concentration of quercetin for 24/48 h, and the cell viability was measured by cell counting kit-8(CCK-8) to determine the IC50 of quercetin (18). (3) The cell cycle distribution and apoptosis rate were measured by Annexin V-FITC/PI double staining flow cytometry(FCM). (4) The protein expression levels of POT1, TRF1, TRF2 were measured by western-blotting. (5) The mRNA expression levels of POT1, TRF1, TRF2 were measured by real-time fluorescent quantitative polymerase chain reaction(RT-qPCR). 2 In vivo: (1) Established AML/NOD/SCID model based on THP-1 cell line in NOD/SCID mice, and treated with optimal quercetin concentration 40mg/(kg*d) for 4 weeks by tail vein injection. (2) We observed the changes of mice survival status, peripheral blood and bone marrow cell morphology and organ histopathology by microscopy before and after treatment with quercetin. (3) The cell cycle distribution and apoptotic rate of spleen cells were measured by Annexin V-FITC/PI double staining FCM. The protein expression levels of POT1, TRF1, TRF2 were measured by immunohistochemistry(IHC) staining.

Results: In this study, we found that quercetin significantly suppressed THP-1 cells proliferation in dose- and time-dependent manner. Treatment with quercetin could significantly increase the expression level of POT1 and TRF1 increased and the protein expression level of TRF2 decreased. The mRNA expression levels of POT1, TRF1, TRF2 were consistent with their protein expression levels, respectively.

Summary/Conclusions: Our results demonstrate that quercetin has anti-leukemia activity. It is mediated by regulating telomere-binding proteins expression of POT1, TRF1 and TRF2. Taken together, our findings support the concept that quercetin is a promising therapeutic strategy for treatment of leukemia.

PB1672
PPARα AGONISTS INHIBIT ADHESION SIGNAL TO ENDOTHELIAL CELLS IN THE DIFFERENTIATION INDUCTION OF HL-60 ACUTE PROMYELOCYTIC LEUKEMIA CELLS.
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Background: All-trans retinoic acid (ATRA) has successfully been used in the treatment of acute promyelocytic leukemia (APL) patients, with a remission rate of greater than 90%. Despite the high cure rates, induction mortality is still a problem in APL. One of the most common causes of death was the differentiation syndrome (DS). The early administration of high-dose dexamethasone at the onset of the first
signs or symptoms of DS is crucial, however specific biological therapies to counteract the syndrome are still not available. Peroxisome proliferator activated receptor gamma (PPARγ) is a ligand-dependent transcription factor and a member of the nuclear receptor superfamily, which is expressed in normal monocytes, various leukemias, and epithelial malignancies. PPARγ is highly induced in differentiating myeloid cells and subsequently contributes to their differentiation. Differentiation induction of APL cells is associated with increased expression of specific adhesion molecules and inflammatory cytokines, which may promote activation, migration, and adhesion of these cells.

Aims: Here, we studied the effect of PPARγ agonists on the adhesion of a human leukemia cell line (HL-60) to endothelial cells.

Methods: Differentiation was determined by an increase in reactivity with the CD11b antibody. For the adhesion assay, the Matrigel transwell system was used.

Results: HL-60 cells were differentiated into macrophage-like cells by a PKC activator, 12-O-Tetradecanoylphorbol-13-acetate (TPA). During the differentiation of HL-60 cells, PPARγ agonists activate TPA-induced CD11b expression. However, PPARγ agonists completely blocked TPA-induced ICAM-1 expression of endothelial cells, which resulted in the inhibition of adhesion of HL-60 cells to endothelial cells. These responses also were reversed by PPARγ antagonist (GW9662), indicating that PPARγ agonists 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.

Acute myeloid leukemia - Clinical

PB1673

IN VITRO DRUG SENSITIVITY TEST IN THE INDIVIDUALIZED ANTI-LEUKEMIA CHEMOTHERAPY FOR THE NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA


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Background: The biological properties, genetic abnormalities of leukemic cells influence on their sensitivity to chemotherapeutic drugs. It is widely known that there can be significant differences both in genetic features as well as in drug resistance profile of individual tumors with the same phenotype.

Aims: The purpose of this study was to analyze the relationship between in vitro chemosensitivity test results using the Cell Titer-Glo assay and clinical response on chemotherapy, and to find the possibility of optimizing the treatment for individual patients according to their actual drug resistance.

Methods: For The Cell Titer-Glo assay, we obtained bone marrow aspirates or peripheral blood samples from 68 patients with newly diagnosed acute myeloid leukemia at the time of initial diagnosis. The following drugs were tested: cytarabine, daunorubicin, idarubicin, fludarabine, etoposide, and methotrexate. We evaluated clinical response and survival outcome according to chemosensitivity of drugs and protein expression.

Results: In this study, in vitro chemosensitivity test with the Cell Titer-Glo assay showed the relationship between chemosensitivity and survival outcome significantly. The 5-year overall survival rates with dichotomized chemosensitivity ofidarubicin (64.6% vs 33.3%, p=0.046), cytarabine (63.1% vs 43.5%, 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 release-free survival rate at 4-year in the patients with low level IC50 than in the high level IC50. However, cytotoxic effect of testing drugs in vitro by the Cell Titer-Glo assay did not show a relationship with complete remission rate after induction and leukemia recurrence rate.

Summary/Conclusions: Although the Cell Titer-Glo assay did not provide the prediction of clinical response of induction treatment, it can be a useful tool in individually optimizing the chemotherapy of patients with newly diagnosed acute myeloid leukemia.

PB1674

PROGNOSTIC IMPACT OF P53 EXPRESSION IN BONE MARROW BIOPSY OF PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Several studies have shown that the presence of the TP53 mutation is related to an unfavorable prognosis in patients with acute myeloid leukemia (AML). However there are few reports on the evaluation of its expression by immunohistochemistry in bone marrow (BM) biopsy.

Aims: To evaluate the expression of p53 in BM biopsy of AML patients at diagnosis and its impact on survival.

Methods: This retrospective analysis included 85 patients with de novo AML diagnosed from January 2005 to December 2015 submitted to BM biopsy at diagnosis. p53 expression was detected by immunohistochemistry, and staining was evaluated using the H-score (range 0-300). The t-test and Mann-Whitney U test were used to detect differences in the distribution of continuous parametric and nonparametric variables, respectively. Overall survival (OS), disease-free survival (DFS) and event-free survival (EFS) were calculated using the Kaplan-Meier method. The log-rank test was used for comparison of survival curves. The interaction between the examined prognostic variables was tested with univariate and multivariate Cox regression analysis.

Results: Median age was 60 years (17-81). There was a predominance of patients >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 leukocytes, ELN risk and p53, all variables remained in the model.
EVI1 expression was related to a lower overall survival in de novo AML.

PB1675

Abstract withdrawn.

PB1676

LONG-TERM FOLLOW-UP OF SALVAGE TREATMENT FOR RELAPSED AML WITH CLADRIBINE, HIGH DOSE CYTARABINE AND IDARUBICIN

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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 clade C (C-CALL) has a more favorable cytogenetic picture in AML as well as an enhancing effect on other cytostatic drugs such as cytarabine ( AraC) and thus may help to overcome resistance mechanisms.

Aims: Therefore, testing the combination of 2CdA, AraC and idarubicin (CAI) seems reasonable. Here we present the final analysis from our single-center phase II trial evaluating the safety and efficacy of CAI in relapsed AML patients after a follow-up of 5 years.

Methods: Patients with relapsed AML after at least 6 months of remission and remission 0-2 were included. Chemotherapy regimen consisted of two courses of 2CdA 5 mg/m²/12 h, d 1-3, AraC 1000 mg/m²/12 h, d 1-3 and idarubicin 8 mg/m²/d, d 1-3. After patients, the prolonged duration of neutropenia especially in course 2 prompted us to change the protocol by 1) application of growth factors from day 15 onwards, and 2) omission of idarubicin from the 2nd course. The primary endpoint was the overall remission rate and safety of CAI.

Results: Because of slow recruitment the study was stopped after 20 patients. The median age was 63 years, 40% were female; 19/20 (95%) patients were included in the first relapse after at least 6 months of CR following 1st line therapy for AML. 1/20 (5%) patient was included with a second relapse. In 14/20 patients cytogenetic data at the time of relapse were available, according to the ELN-risk-group 2017 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% of patients). After the first course, CR/CRi was achieved in 60% and PR in 10% of patients. Median duration of neutropenia grade 3 or 4 was 14-16 days. The rate of grade 3 or 4 non-hematologic toxicity was infection seen in 85% of courses. Nausea occurred in 30%, hepatotoxicity, mucositis and diarrhea in 11% of courses. Cardiac or renal toxicities grade 3/4 were not observed. Two patients (10%) died due to infection. Six patients received a second course of CAI/CA. Altogether, 6 patients were refractory. Nine patients (48%) proceeded to allogeneic stem cell transplantation after induction therapy with CAI. Of those, 4 patients are still alive and free of leukemia and one patient died in CR 88 months after salvage-therapy accounting for a 5-year survival rate of 55%.

Summary/Conclusions: Combination therapy with CAI in relapsed AML patients is feasible and induces good response rates. Combined with allogeneic stem cell transplantation, long-term survival can be achieved. However, 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

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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 integration 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. EV11 prognostic significance in AML remains to be confirmed in other populations. Furthermore, because the selection protocol and cutoff value selection methodologies differed among studies, the threshold for defining EVI1 high expression remains obscure, which hinders its clinical routine application.

Aims: We investigated the prognostic impact of EVI1 transcript levels in Chinese adult intermediate cytogenetic risk AML (iC-AML) patients who received chemotherapy only in a single center. The appropriate cutoff values for grouping EVI1 expression were also evaluated.

Methods: A total of 191 adult patients receiving chemotherapy only were included in this study. They were diagnosed as iC-AML according to morphology, immunophenotyping, cytogenetics and molecular biology. Their bone marrow samples were collected at diagnosis. Real-time quantitative PCR was performed to test EVI1, MLL partial tandem duplicate (MLL-PTD) and WT1 transcripts, and their transcript levels were calculated as the percentage of target transcript copies/ABL copies. NPM1 mutations and FLT3 internal tandem duplication (FL3-ITD) were individually screened by real-time quantitative PCR and qualitative PCR, respectively. Patients were simultaneously tested EVI1, MLL-PTD and WT1 transcripts. All participants 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% was significantly associated with lower 2-year relapse-free survival (RFS), disease-free survival (DFS) and overall survival (OS) rates in the entire cohort (P=0.0003, 0.0017 and 0.0009), patients with normal karyotypes (n=148, P=0.0032, 0.0047 and 0.0007) and FLT3-ITD (-) patients (n=150, all P<0.0001). Multivariate analysis showed that EVI1≥1.0% and FLT3-ITD (+) were independent adverse prognostic factors for RFS (Table 1), DFS and OS in the entire cohort. In addition, patients with EVI1 between 1.0% and 8.0% had 2-year RFS rates similar to those with EVI1>.8% (P=0.16), and both patient groups had significantly higher RFS rates than those with EVI1<1.0%.

Table 1.

Summary/Conclusions: EVI1 transcript levels at diagnosis could further stratify adult iC-AML, and high EVI1 expression predicts poor outcomes in patients receiving chemotherapy only. The optimal cutoff value which best differentiates patients is different from the normal upper limit.

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PB1678

EFFICACY AND SAFETY OF DECITABINE IN ELDERLY AML PATIENTS: A REAL LIFE MULTICENTER EXPERIENCE OF THE NETWORK RETE EMATOLOGIA LOMBARDIA

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Background: The optimal treatment decision in older patients (pts) with AML remains controversial, especially in patients pts with comorbidities, non-fit to intensive therapy or with AML adverse biologic features. Recently decitabine was approved in Italy in AML pts unfit to chemotherapy aged >65 years (y) and could be adopted in a population based setting.

Aims: To evaluate efficacy and toxicity of decitabine in a consecutive series of elderly AML pts (no M3), considered unfit to chemotherapy (CT) according to Ferrara et al (Leukemia, 2013) and treated at 6 centers of the Hematological Lombardy Network (REL).

Methods: Between Dec 2015 and Dec 2016, 46 (F/M: 22/24) newly diagnosed AML pts were included in the study according to the CT excluding criteria for decitabine in elderly AML pts unfit to chemotherapy aged >65 (y) and potentially eligible for decitabine. Median age was 76 y (69-85), ECOG performance status (PS) was ≥3 in 10.8%. According to “fitness”, 41 pts (89%) were defined unfit to intensive CT, 1 frail and 4 fit. Unfitness causes were age >75 (58.5%), PS ECOG≥3 unrelated to leukemia (12.2%), and comorbidities (29.3%). AML was “de novo” in 25 pts (54.3%), therapy-related in 3 and secondary to antecedent hematological disorders in 18 pts. WBTC count at diagnosis was 4.4 x 10³/μL (0.46 to 63), marrow blasts were 51% (<30%) in 19.5% of pts. Karyotype (K) was normal (NK) in 43%, t(8;21) in 4.5%, intermediate in 20.5%, adverse (adv) in 32% of
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 51.3% and 32%, respectively. Partial response (PR) and hematological improvement were achieved in 5 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). Relapse/disease progression was observed in 42 of responders. ORR was 21.4%, 47.3% and 77% in adv, NK and intermediate K, respectively (P=0.0289). After a median follow-up of 6.5 months, median survival was 8.4 months and projected OS at 1 and 2 y was 43%+9 (SEM) and 30%+12 (SEM). Treatment was fairly well tolerated except for a high incidence of infections (46 episodes in 231 cycles) particularly during the first 3 cycles (29% vs 11%) (p 0.0072). Pneumonia was the most frequent infection (46%), followed by sepsis (28%). It was more frequent during the first 3 cycles [14% vs 4%: p 0.012] when 44% of cases were of suspected fungal origin (3 probable aspergillosis and 4 possible IFI). Death occurred in 24 pts (52.2%); 12 (50%) of disease progression, 1 of early CNS hemorrhage and 11 (45.8%) of infection. In the first 3 months, infections were responsible for 46.7% of deaths. Pulmonary IFI were fatal in 57% of cases. These figures are higher than those reported by Cashen (JCO 2010) where the frequency of pneumonia was 11%.

Summary/Conclusions: These preliminary data confirm, in a population based setting, the high efficacy of decitabine and its longer time to response (more than 3 months) compared to CT. However infections complications were more frequent than expected and often fatal, particularly during treatment. Since pneumonia, especially IFI, was the major cause of death, the adoption of routine antimicrobial prophylaxis may be considered in order to reduce early mortality and further improve the results.

PB1679
CLOFARABINE, CYTARABINE AND MITOXANTRONE FOR RELAPSED OR REFRACTORY ACUTE MYELOID LEUKAEMIA – INTERIM RESULTS OF A PROSPECTIVE PHASE 2 STUDY
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Background: In unselected patients with acute myeloid leukaemia (AML) in first relapse or refractory to primary daunorubicin / cytarabine therapy, complete response (CR) rate is merely 20 - 30%. In patients <60-years old, CR rates of about 55% may be achieved.

Aims: We tested in a multicenter prospective phase 2 study the efficacy and safety of clofarabine, cytarabine and mitoxantrone (CLAM) in AML patients in first relapse or refractory to first-line daunorubicin / cytarabine induction therapy. Next-generation sequencing of a myeloid panel of 67 genes was performed. Re-induction CLAM comprised clofarabine (40mg/m2/day, days 1-5) and mitoxantrone (12mg/m2/day, days 1-5). Bone marrow assessment was done on day 28 using standard criteria. Treatment toxicity was evaluated using the Eastern Cooperative Oncology Group Common Toxicity Criteria (ECOG-CTC). Survivals were determined using Kaplan Meier method. The primary outcome was the response on day 28. Secondary outcomes included toxicity, leukaemia-free and overall survivals.

Results: In this interim analysis, 24 patients (14 men, 10 women) with a median age of 44.5 (19-66) years were treated. Karyotypic and genetic profiles were: normal karyotype (N=8) (NPM1 mutant, N=1); FLT3-ITD WT, N=8; (8;21)(q22;22) (N=4) (KIT D816V mutant, N=1), inv(16)(p13.2;q22) (N=16) (p13.2;q22) (N=1) (KIT D816V mutant, N=1), del (11)(p13); t(9;11)(p21;q31) (N=1), trisomy 13 (N=1), near-tetraploidy (N=1), and complex karyotype (N=1). Twenty patients (83.3%) responded (CR, N=16; CR with incomplete hematopoietic recovery, N=4). Eight responding patients underwent allologeneic haematopoietic stem cell transplantation. Grade 3/4 haematologic toxicity was observed in 17 (70.8%) and 2 (8.3%) patients respectively. Grade 1/2 rash was observed in 4 patients (20%). Cardiotoxicity or treatment-related mortality was not seen. With a median of follow-up of 4 (1-32) months, 6 patients relapsed. The 12-month overall and leukaemia-free survivals were 81.7% and 66.8% respectively.

Summary/Conclusions: CLAM resulted in a high CR rate for AML in first relapse or refractory to first-line induction therapy, which was associated with an acceptable toxicity profile.

PB1680
FATAL EVOLUTION IN THE FIRST 96 HOURS OF PATIENTS DIAGNOSED WITH ACUTE LEUKAEMIA: ANALYSIS OF A SERIES OF 346 CONSECUTIVE CASES OF ACUTE LEUKAEMIA IN A SPANISH HOSPITAL
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Background: The very early death of a newly diagnosed acute leukemia (AL) patient is very frustrating, and there are very few published works (except for the case of acute promyelocytic leukemia, APL) analyzing this circumstance and the features of these patients.

Aims: Our objective was to study the main characteristics of patients with acute leukemia who died within the first 96 hours after diagnosis in our centre in the last 15 years.

Methods: We studied all cases of acute leukemia diagnosed in our institution between April 2002 and January 2017, focusing on the analysis of those who died within the first 96 hours after diagnosis. In this subset of patients, we collected data concerning clinical presentation, hemogram, biochemical parameters, coagulation status, performance of a bone marrow aspirate, acute leukemia subtype, started therapy, initiation or not of induction chemotherapy, time elapsed from diagnosis to death (hours), and cause of death, among others.

Results: A total of 346 consecutive cases of acute leukemia were recorded in this period of time: 222 of acute myeloid leukemia (AML, 64%) and 124 of acute lymphoblastic leukemia (ALL, 36%). Thirty-three patients were diagnosed of acute promyelocytic leukemia (15% of all AML). Those patients who died in the first four days after the diagnosis were only seven (2%), with a median of 45 hours. In the range 21-96 hours, 35 clinical and analitical clinicals are shown in the Table 1. They were 5 men and 2 women with a median of 57 years (range 22-91). Two of the seven patients had an APL (6% of all diagnosed APL). All patients showed leukocytosis, but hyperleukocytosis was only recorded in 2/7 patients, and severe thrombocytopenia (Plt ≤ 20 x109/L) in 3/7. There was possible of bone marrow aspiration only in 4/7 cases. Coagulopathy was detected in four of six patients, including criteria for disseminated intravascular coagulation (DIC) in three cases. The exitus took place in the Intensive Care Unit in 5 cases, while it occurred in the Hematology facility in two.

Table 1.

Summary/Conclusions: In our experience, about 2% of patients with acute leukemia die within the first 96 hours after diagnosis (including 6% of APL). Clinical and analytical features of this subset of patients are very heterogeneous, although AML clearly predominate on ALL. More extensive and multi-center studies are needed to deepen into the circumstances conditioning this early fatal course of the disease.

PB1681
PRIMARY POSACONAZOLE PROPHYLAXIS IN ACUTE MYELOID LEUKAEMIA - A SINGLE CENTER REAL LIFE EXPERIENCE
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Background: Invasive fungal infections (IFI) are a major cause of mortality and morbidity in acute myeloid leukemia (AML) patients receiving remission induction therapy, and relapsed/refractory AML patients. Posaconazole prophylaxis has shown the greatest benefit in preventing IFI in AML. Aims: We present the data of our real-life experience in AML patients under PP. Methods: We have retrospectively reviewed the data from 82 AML patients
receiving 105 cycles of chemotherapy between June 2012 and December 2016 in Manipur University Postdoc Research and Training Hospital. Median patient age was 50 years (18-73); and there was no significant gender difference (38 females vs 44 male (46% vs 54%). All patients had active disease, 78 (74.3%) of them received 3+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±3 (1-68) days. This duration was 29.7 days (16-50) in patients receiving prophylaxis until marrow recovery and 8.9 (9-34) days IFI under prophylaxis and 12.7 (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 was due to adverse events in 6 cycles (5.7%), and due to other reasons (diarrhea, intolerance of oral medication, recurrent high grade fever, death) in 39 cycles (37.1%). IFI incidence under effective posaconazole prophylaxis was 28.1±18.64. Total clinical failure rate was 60% (63/105). IFI was diagnosed with pulmonary nodules in 12 of 18 patients (66.6%, EORTC-MSG: possible/probable) with galactomannan positivity in 3 patients (16.6% EORTC-MSG: probable), and with fungal culture in 3 patients (16.6%; EORTC-MSG: proven). Data from 70 patients were available for mortality analysis. In patients receiving effective posaconazole prophylaxis, all-cause mortality rate at day 100 was (8/44; 20.4%) significantly lower than patients unable to continue possible/probable prophylaxis (12/28; 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 efficacy and safety of posaconazole prophylaxis. Although our IFI rate was comparable to other real-life data, our clinical failure rate was slightly higher. This is probably due to compliance issues, since in many chemotherapy cycles (37.1%) posaconazole was discontinued due to “other reasons” such as drug intolerance. Although not as effective as in the clinical trials; our data still supports the use of posaconazole prophylaxis in high risk AML patients.

PB1682

CLINICAL AND PROGNOSTIC VALUE OF FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA PATIENTS IN ROUTINE CLINICAL PRACTICE – SINGLE CENTER EXPERIENCE

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Background: Detection of FLT3 gene mutations in acute myeloid leukemia (AML) now recognized as an unfavorable factor that affects the disease course, emerging the risk of relapses and overall survival (OS) shortening. Although about 30% of AML patients harbor one of the FLT3 gene lesion, at present there are no internationally standardized assays to quantify FLT3 mutation burden and no results of randomized clinical trials intended to individualize AML treatment based on FLT3 status. Some hematologists advocate to allo-SCT as consolidation in FLT3 ITD+ patients, but this way could be hard in frail and old patients as well as 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 in the cohort 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 (20-86 years). To determine FLT3 gene mutations we used the method of polymerase chain reaction (PCR) with subsequent restriction. FLT3 gene mutations were classified as internal tandem duplication (FLT3-ITD) and point mutation in the “A-loop” (FLT3-TKD). Statistical analysis was included Kruskall-Wallis ANOVA and Kaplan-Meyer curves.

Results: We observed two distinct FLT3 gene mutations rates: FLT3-ITD - 22.6% (45/199), FLT3-TKD 5.5% (11/199), FLT3-ITD and FLT3-TKD in combination 1.0% (2/199), other 70.8% (141/199) patients had no mutations (FLT3-). CBC data at the time of diagnosis were as follows (median [max-min]): - FLT3-ITD: Hb 9.7 (3.7-13.0) g/dl, WBC 40.3 (0.6-400.0) x 10⁹/L, blasts 80% (21-100), platelets 60 (2-140) x 10⁹/L; - FLT3-TKD: Hb 10.2 (5.9-12.8) g/dl, WBC 62.4 (1.7-362.0) x 10⁹/L, blasts 68% (23-100), platelets 55 (12-115) x 10⁹/L; - FLT3-ITD+TKD: Hb 5.8, 8.4 g/dl, WBC 37.0, 157.0 x 10⁹/L, blasts 65%, 86%, platelets 38, 186 x 10⁹/L; - FLT3: Hb 9.0 (2.8-14.0) g/dl, WBC 12.9 (1.0-260.0) x 10⁹/L, blasts 64% (20-103), platelets 63 (1-334) x 10⁹/L; Significant differences across the groups were seen only in W/L and blasts. Chromosomal aberrations were revealed in 38% of FLT3-ITD, 64% of FLT3-TKD, none of FLT3-ITD+TKD and 51% of FLT3- patients. All patients received chemotherapy (7±3, 5±2, HAM). Transplantation of hematopoietic stem cells (SCT) was performed in 28 (allo/auto 17/11) (14%) patients: FLT3-ITD allo-3; FLT3-TKD allo-1, auto-1; FLT3-ITD allo-1, auto-10. We found significant (p=0.00024) differences regarding to OS between FLT3-ITD, FLT3-TKD and FLT3- patients (Figure 1). Median survival times were: 5.1 months for FLT3-ITD, 7.1 months for FLT3-TKD and 13.0 months for FLT3- patients.

Summary/Conclusions: We confirmed the role of FLT3 gene mutations as an unfavorable factor for AML patients in routine clinical practice by own experience. The investigation of qualitative assessment potential and target therapy value especially in SCT ineligible FLT3 gene mutations positive patients has of great value for AML management.
Results: In the debut of AL prior to the CT in all 66 (100%) patients the increased activity of total NOS in 3.8 times compared with the norm (p=0.001) was noted, with simultaneously reduced concentration of [NO2]- in 1.5 times relatively normal values (p<0.05) (Table 1). As a result of two CT courses of remission induction in patients of group II the trend to decrease the total NOS activity compared with its level before treatment was observed. At the same time the significant decrease of [NO2]- in 1.8 times relatively normal values (p<0.01) and a trend to lower their content in 1.2 times compared with the data before treatment (p>0.05) was noted. These changes constitute the violation of NO-dependent vasodilation mechanism and endothelial dysfunction intensification. Provided achieving low cumulative dose of AA in patients of group II on the background of AC prevention with L-arginine showed a significant decrease in 1.9 times the total NOS activity (<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.

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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 substrate production, accompanied by endothelial dysfunction impairment. The additional appointment of L-arginine on the background of CT can restore synthesis of NO and, respectively, the mechanism of NO-dependent vasodilation, thus reducing the risk of early anencephaly cardio toxicity 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 whenever possible rather than as myelodysplastic syndrome. The 2016 revision of the WHO reclassified erythroid/myeloid subtype (a case with ≥50% BM erythroid precursors and ≥20% myeloblasts among non-erythroid cells) to MDS category based on the close biological and genetic relationships between them. Aims: The aims of this multi-center study were to characterize clinical characteristics and treatment outcomes in patients with newly diagnosed acute erythroid leukemia.

Methods: Clinical data from newly diagnosed M6-AML patients between 2002 and 2012 at 11 academic centers were retrieved from the electronic registry 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-bandinge technique. Patients were classified according to the UK MRC cytogenetic risk criteria and the International Prognostic Scoring System (IPSS) risk groups for MDS based on karyotypes. Survival curves were analyzed using the Kaplan-Meier method and compared with a log-rank test. A p-value <0.05 was considered statistically significant.

Results: A total of 84 patients with AEL (M6-AML) as defined by 2008 WHO classification criteria were included in this study. The median age at diagnosis was 55 years with following distribution: age ≤ 49, 34 patients (40.5%); age 50–59, 17 (20.2%) patients; 60 – 69, 19 (22.6%) patients; age ≥70, 14 (16.7%) patients. There were 50 (59.5%) males and 34 (40.5%) females. Median hemoglobin, white blood cell count, and platelet count were 8 g/dL, 3.69 × 10⁹/L, and 58 × 10⁹/L, respectively. Peripheral blood blasts were observed in 55 (65.5%) patients. Cytogenetic 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 (9;22)(q34;q11.2). Cytogenetic risk group based on 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).

Figure 1.

Summary/Conclusions: Patients in this study were younger than previous studies. The most common aberrations in chromosomes are trisomy 8, followed by numerical changes in chromosome 5 and 7. The median in total patients was 21 months with many patients received intensive treatment, including HSCT in 45.2% of patients. We also confirmed that patients with poor-risk karyotypes had very poor median OS of 7 months. Therefore, we suggest that although erythroid/myeloid subtype is similar to the MDS with excess blast, treatment decision might be carefully considered according to the karyotype risk.

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-
rical condition that limits the possibility to conduct large prospective clinical studies. All publications present small retrospective data and case reports. Most of them conclude that pregnancy doesn’t affect the prognosis of acute leukemia.

**Aims:** To assess the pregnancy, as independent prognostic factor, in non APL AML-patients (pts), prospectively treated within Russian AML multicenter studies.

**Methods:** From 1990 to 2017 y the Russian Acute Leukemia study group has treated 33 with de novo AML pregnant women (Me-27 (21-42) yrs), AML was diagnosed in the 1st trimester in 1 woman (3%) , in the IIInd 15 (45,5%), in the IIIrd 17 (51,5%). Molecular genetic risk was estimated in 27/33 pts: 52% (n=14) were referred to the intermediate risk group and 48% (n=13) to the poor prognosis. High risk group comprised complex karyotype (n=5), t(7;11)(q21;q22), translocations inv(11)q23, 1 pt - inv3;7 and 1 pt - AML with myelodyplasia-related changes, normal karyotype and FLT3-ITD.

In 1 pt at the 1st trimester medical abortion was conducted and 11 women delivered at the gestation age of 34-40 weeks before chemotherapy (CT). 21 pregnant women received CT, that was started at 23 (14-32)nd weeks of gestation. Classical 7+3 was applied in all of pts: either with daunorubicin (45-60 mg/m²), or mitoxantrone (10 mg/m²), or idarubicin (12 mg/m²) according to the treatment study-protocol.

**Results:** As our data show, AML in pregnancy is characterized by high prevalence of unfavorable cytogenetic abnormalities (48%), that is substantially different from AML in non-pregnant women of the same age (11,5%) (p=0,006) [Blood 2016;128;22.p5171]. 1 pt died before CT due to septic shock, 2 pts – in induction CT now. 2 pregnant women died due to severe infections in aplasia during induction (5,7%). So, induction results were evaluated in 30/33 pts: CR rate - 73,3% (22/30): after the 1st course CT 26 in 6 months after CR, 4 pts relapsed after allo-BMT and 1 woman remained with refractory AML after allo-BMT. Our results demonstrated rather low 1y OS and DFS (10,4% and 10,4%), high CR rate (100%) among the 67 patients (75%), with a median overall survival of the whole cohort was 115 days , with a median event free survival of 111 days. Among the responding patients, 16 (24%), underwent allogeneic bone marrow transplantation; in these selected patients, median overall survival was 185 days.

**Summary/Conclusions:** These results suggest that the clofarabine-ARA-C regimen was able to induce a response in about one third of this particularly poor prognostic category of patients, with a safety data consistent with previously reported salvage therapies. Nevertheless, long term results remain still and completely unsatisfactory. Further studies, with different combinations or in more selecting conditions, are warranted.

**PB1687**

**PRESENCE OF MULTIPLE DRIVERS IN THE SELECTION OF HIGH AND LOW INTENSITY CHEMOTHERAPY IN AML**

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**Background:** Data on the key drivers of initial treatment choice for patients diagnosed with acute myeloid leukemia (AML) in the United States is limited. The use of age as a selection driver of induction therapy is well established; however, there is limited data and a knowledge gap about additional factors driving treatment selection.

**Aims:** This analysis explored the key physician drivers, which led to the selection of high- and low-intensity induction therapy in AML patients.

**Methods:** Data from the Adelphi AML Disease Specific Programme, a real-world, cross-sectional survey conducted between February–May 2015, was analyzed. A total of 61 hematologists/oncologists provided attitudinal information about their management and treatment choices for AML patients via survey. Each physician was provided a pre-specified list of 16 patient characteristics. Via two separate questions, they were asked to select those considered important when choosing high and low intensity chemotherapy for their AML patients. Characteristics were analysed descriptively and ranked based on the frequency of mention from highest to lowest.

**Results:** The top three drivers for decision making when selecting high and low intensity treatment were: patient age, performance status and presence of comorbidities. More than 60% of physicians would prescribe high-intensity treatment for patients aged under 65, with a good performance status or with no comorbid conditions. Over half of physicians would consider those who are eligible for a stem cell transplant or have a mutation in the CEBPA gene to be eligible for high-intensity chemotherapy (Table 1). Low-intensity chemotherapy was considered by more than 60% of physicians as being the most appropriate treatment for patients aged 65 or older and had previous or current exposure to radiation/chemotherapy in the past.

**Table 1. Top 5 patient characteristics considered by physicians when choosing high- or low-intensity treatment in AML.**

**Summary/Conclusions:** Irrespective of treatment intensity, patient age, performance status and the presence of comorbidities are the top three drivers of treatment selection for physicians. In addition to patient age, identification of the other key drivers for therapy selection and the physician awareness of them is critical to ensure patients receive the most appropriate therapy. This improved awareness could also lead to better communication tools for patients and improve shared decision-making.
Background: IRAIN which is produced from the insulin-like growth factor type 1 receptor (IGF1R) imprinted locus is a newly identified IncRNA. There are very little knowledge about the specific role of this IncRNA in 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 were analyzed in bone marrow specimen of AML patients (n=76) and healthy individuals (n=18).

Results of detection in the ITD assay was found to be remarkably decreased in AML patients compared with healthy individuals (p= 0.02). Significant IRAIN down-regulation was observed in all FAB types except for the M3 (p = 0.11). When we analyzed the expression level of IRAIN in different cytogenetic subtypes of AML patients the statistically down-regulation of IRAIN was observed only in poor prognosis AML group (p=0.008).

Summary/Conclusions: Our results suggest that down-regulation of IRAIN IncRNA might play a role in the AML development and hence may be a potential prognostic factor and serve as therapeutic target for AML treatment.

PB1689

PERFORMANCE OF THE LEUKOSTRAT® CDX FLT3 MUTATION SIGNAL RATIO ASSAY TO DETECT INTERNAL TANDEM DUPLICATION AND TYROSINE KINASE DOMAIN MUTATIONS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Background: Acute myeloid leukemia (AML) in general has a poor prognosis. Assessment of the mutation status of the FLT3 (fms related tyrosine kinase 3) receptor gene in AML is the most important prognostic indicator of disease outcome, which is often substantial, as many studies in AML have shown that the presence of FLT3 activating mutations portends a poor prognosis. The 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 activation of FLT3-ITD TKD mutations are caused by nucleic acid substitutions and/or deletions that result in a change in the amino acid sequence in this highly-conserved catalytic center. TKD mutations, such as D835 and I836 substitutions and deletions, result in constitutive phosphorylation 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 peak. A FLT3 mutation was detected (and reported as positive) if the mutant:WT type SR met or exceeded the clinical cut-off of 0.05. Proprietary software calculated the SR and reported positive or negative. Clinical specimens were de-identified by LabPMM in San Diego. DNA from twenty specimens was tested by three laboratories: LabPMM LLC in San Diego, LabPMM GmbH in Germany and LabPMM Gk in Japan.

Results: The analytical performance of the LeukoStrat® CDX FLT3 Mutation Assay was evaluated using contrived LDB samples, with known FLT3 mutations. For limit of blank (LoB), the SR was 0.00 in the ITD assay and 0.00 to 0.01 in the TKD assay, which is well below the clinical cutoff SR of 0.05. The limit of detection of 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 relative cell type and number of operators. 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 different continents provides evidence that the Invivoscribe® LeukoStrat® CDX FLT3 Mutation Assay is an internationally standardized assay.

PB1690

CLINICAL FEATURES AND OUTCOME OF PATIENTS WITH CORE BINDING FACTOR ACUTE MYELOID LEUKEMIA

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Background: Acute Myeloid leukemia is classified into different prognostic groups according to their cytogenetic profile; AML with t (8; 21) or inv(16) i(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 16i(16; 16) and del16q22. Induction treatment: Daunorubicin 45 to 90 mg/m2 day (d1-d3)+Cytarabine 100 mg/m2 (d1-d7) (with progressive doses if major leukocytosis). Assessment between d 21 and d 28 by bone marrow analysis: If failure a study. To assess the performance of the Invivoscribe® LeukoStrat® CDx FLT3 Mutation Assay.

Results: From 2010 to 2016, cytogenetic analysis was performed in all cases of AML of which 58 cab1 (18.6%) of LAB - CBF were diagnosed. The male to female ratio was 0.5; average age 47 years (17-72); t (8; 21) was found in 28 pts (16 M, 12F); inversion (16i) (p13.11:q22.1) t(16i)(16i); p13.11:q22.1) 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). AML2 (1) and 3 AML. For inv(16), 24 pts were analyzed. 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 before engraftment). Median overall survival for inv(16): 11 months vs 15 months for t(8; 21) (p=0.87).

Summary/Conclusions: In our study, the frequency of the CBF AML is closer than those described in another Algerian study and literature: 18.6% vs 15.4% and 20% respectively; a slight predominance of the inv 16 or t (16; 16) identical translocation 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. 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 utilises the latest strides made in web technologies to respond to the varying screen sizes of devices, and display suitably sized graphs and gating information accordingly. Collaboration between parties is facilitated - a lab technician running the sample can upload the sample and communication can take place immediately and can then be instantly shared via a web URL. No sensitive file data is displayed within the platform. All data transfer happens via SSL encryption.

Web app is available at https://www.redmatterapp.com

Figure 1.

Summary/Conclusions: The latest web technologies can be effectively harnessed to enhance flow cytometry analysis and allow for faster, more accessible and more collaborative analysis. Within the field of haematology in particular, this opens up the option of remote diagnosis - a haematologist need not be in the lab, or even in the same country, to deliver a diagnosis.

PB1692

FLAG-IDA IN THE TREATMENT OF ACUTE LEUKEMIA: SINGLE-CENTER EXPERIENCE

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Background: A variety of different treatment regimens have been studied in an effort to improve outcomes of patients with relapsed or refractory acute myeloid leukemia (RR-AML), there appears to be no single superior approach. Spanish groups usually use the FLAG-IDA protocol (Fludarabine 30 mg/m2 days 1-4, Idarubicine 12 mg/m2 days 1-3, ara-C 2 mg/m2 days 1-5) 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 and average age of 53.4 years (DS±23.3). We treated with this protocol mostly patients with relapsed or refractory acute myeloid leukemia (RR-AML) (primary refractory or resistant AML as defined by not achieving complete remission after 1 cycle of intense induction therapy); 60% (n=38) of patients had a RR-AML, 37% (n=23) of them were relapsed AML due to 23% (n=15) refractory AML. 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 total registered patients. We can see in the overall survival curve (picture 1) that most patients died first months after treatment, after that patients remain alive and we achieve a plateau. The median overall survival was 82 days (standard deviation: 25 days). 10 patients were alive when we analyzed the data (Figure 1).

Summary/Conclusions: Most AML patients ultimately die from their disease. In our case serie none died by any other cause. We had a similar response rate, mortality and overall survival that other groups in our country. Despite a variety of salvage therapy options, like FLAG-IDA protocol, prognosis in patients with RR-AML is generally poor and treatment is very complex.

PB1693

BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASMS - UNUSUAL PRESENTATIONS AND UNFAVOURABLE OUTCOMES

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare hematological malignancy with an aggressive clinical course. Most patients (pts) with BPDCN have skin lesions and involvement of the peripheral blood, bone marrow, and lymph nodes. Very few cases have been described with lack of skin and or bone marrow manifestations at the time of diagnosis.

Aims: To characterise the clinical presentation and clinical outcomes of a cohort of consecutive patients with a rare blastic plasmacytoid dendritic cell neoplasm in a single institution.

Methods: Patients diagnosed with BPDCN at the National Hematology Hospital between 2010 and 2016 were retrieved from the database. The diagnosis was confirmed by morphology and immunophenotyping by flow cytometry and/or immunohistochemistry, according to 2008 WHO Classification of Hematopoietic Neoplasms. The relevant clinicopathologic features were reviewed.

Results: We identified 8 adult patients at a median age of 70 years (range: 37-84 years) with a male:female ratio of 6:2 (75%:25%) and only 1 child. Mean values of blood cell counts were as follows: WBC 5.10^9/L; hemoglobin 99 g/L; platelets 116.10^9/L; LDH was generally elevated with a mean of 962.8 U/L. At diagnosis 50% of the pts had skin involvement. Skin was involved in 50 pts, skin involvement was more prominent in less differentiated subtype (29% pts) than in blastic (25% pts) and CD56+ (67% pts) than CD56- (16% pts) BPDCN. The most common sites of skin involvement were testis, bronchial wall, stomach and periorbital soft tissues, however, only the latter one case presented with a leukemic picture. Biopsies revealed diffuse, monomorphous infiltrate of medium-sized blast cells with irregular nuclei, fine chromatin with ≥1 nucleoli, scant and agranular cytoplasm, without angioinvasion or coagulation necrosis. Immunophenotype generally demonstrated CD45+, CD4+, CD56+, CD123+. No standard therapies were applied. Patients received CHOP or HyperCVAD or AML-induction therapy. However, response rates in adult patients were low and the mean OS was 2.6 months (ranging from early deaths before any treatment could be initiated to 10 months).

Summary/Conclusions: BPDCN is a rare aggressive disease that typically affects elderly patients. The most commonly affected non-hematopoietic organ is the skin, however any other organ or tissues can also be involved. Response to therapy if any is relatively short and long-term prognosis is poor despite of the site of presentation. Larger scale studies are warranted to understand the pathophysiology of the disease and to find optimal management.

Acknowledgements: Partial support by the National Science Fund.

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: In our case serie none died by any other cause. We had a similar response rate, mortality and overall survival that other groups in our country. Despite a variety of salvage therapy options, like FLAG-IDA protocol, prognosis in patients with RR-AML is generally poor and treatment is very complex.
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 1:2.2 and median age at diagnosis 5.5 years (Min: 1.3 months, Max: 13.8 years). 12 patients were excluded from further analysis for not being able to complete induction therapy. Donor’s syndrome, 7.7% (11 of 143) had concomitant malignancies. 85.7% (120 of 134) were CNS-1, 24.7% (20 of 73) had MLL gene rearrangement, 21.2% (14 of 66) were positive for TELAMLRT/TEL/RUNX1/TEN1 and 22% (13 of 59) had PML/RAR (+). Trisomy 4, 10 or 17 was not seen among any of 13 patients tested. Most commonly observed FAB classification was M-5 (23.5%, 24 of 102) followed by M-2 (18.6%). 27.3% (39) were Low Risk, 43.4% (62) Intermediate and 29.4% (42) High Risk, 43.3% (58 of 134) received HSCT.

Results: Our CR-1 rate was 93.7% (143 of 140) with 100% in Low Risk, 95.2% Intermediate Risk and 85.7% in High Risk patients (P-Value: 0.023), requiring 1-3 cycles of chemotherapy with a median time of 1.3 months. Treatment Failure was observed in 6.3% (9 of 143). Relapse rates was 38.8% (52 of 134). Most common site of relapse was bone marrow (75%, 39 of 52). PML/RAR (P-Value: 0.044), Post-Induction BM Classification M-3 (P-Value: 0.034) and AML High Risk (P-Value: 0.003) were found to be significantly associated with Relapse. Age at diagnosis, or Time to CR-1 were not found to have any association with relapse. 51.9% (27 of 52) who relapsed, went for HSCT. With a median follow-up of 68.8 months, five year overall survival for our cohort of patients was (0.567±0.046); significantly poor (P-Value: 0.001) in relapsed (n=52, 0.197±0.051) compared to non-relapsed (n=82, 0.862±0.041); resulting in a five year overall survival of 0.472±0.044. Among relapsed group (n=52), five year overall survival was significantly better (0.160±0.073) for those who received HSCT (27) than who did not (n=25, 0.114±0.073; P-Value: 0.029). Five year overall survival was also significantly better for Non-Relapse group (n=31, 0.828±0.070) compared to relapsed patients (n=27, 0.160±0.073; P-Value:0.003) for which HSCT was administered (n=38).

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 been described to be responsible for solid tumors chemoresistance, invasion, metastasis and relapse. The role of HIFs in leukemias has not been established so far. First reports of poor outcomes of allogeneic treatment linked with overexpression of HIF-1α 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 HIF-s alpha expression in acute myeloid leukemia cells (AML) influences the prognosis in patients with AML.

Methods: We analyzed 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 Continine 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 ± (33.32) and 8.48 ± (11.63) before and after chemotherapy respectively (p = NS); In NR group the values were 11.74 ± (22.6) and 24.01 ± (33.68) respectively (p < 0.001) (Figure 1). The Cox analysis revealed HIF-2 alpha positive blasts in blood after chemotherapy to be proportional to death probability (p=0.0036) (Figure 2).

Summary/Conclusions: We are aware our results are preliminary. But if they are confirmed it will be very interesting to determine the role of HIF-2 alpha inhibitors in improving the prognosis and survival in human AML.
consolidation chemotherapy was postponed, relapsing without reach the already planned transplantation. At the bone marrow karyotype was 46XY, t(9;22)(q34;q11) and the molecular biology showed the presence of p190 e1a2 and e6a2 isoforms and FLT3-ITD mutations with a low mutant allelic burden (Figure 1B). Salvage chemotherapy was then performed, allowing at this time to obtain disease remission and further allogeneic transplantation. Neverthe-
less, the patient died 5 months later for transplant complications. qRT-PCR assays performed in diagnosis sample showed the main clone FLT3-ITD accompanied by subclones with p190 e1a2 and e6a2 isoforms. These data indicate a clonal selection process and the expansion of a resistant clone with p190 e6a2.

Figure 1.

Summary/Conclusions: The atypical p190 e6a2 transcript seems to be associ-
ated 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 REFRACTIVE AML: A 2-CENTERS RETROSPECTIVE STUDY

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ogy Unit, Padova, 2Division of Hematology and Bone Marrow Transplantation, Azienda Sanitaria-Universitaria Integrazione, University of Udine, Udine, Italy

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 is limited.

Aims: To define efficacy and feasibility of hypomethylating agents (HMA) as salvage chemotherapy in patients with 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 ELN 2010, 11 were inter-
mediate 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). 28% of patients underwent allogeneic stem cell transplantation after HMA therapy. Median OS was 197 days from the starting of HMA and median EFS was 70 days. Median OS in patients with refrac-
tory 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 with relapsed disease was 198 days (p=0.039). We did not find significant differences between transfusion needs before and after sal-
vage therapy but this could be due to the small size of our sample.

Summary/Conclusions: HMA showed efficacy and a considerable OS in our patients. In our cohort refractory patients were almost all refractory to HMA too, and their OS was dismal. So HMA could be a good clinical option in a selected population of relapsed patients, especially in those not suitable for allogeneic bone marrow transplantation, in whom the prognosis is generally extremely poor. Further studies are needed to determine which are the cytogenetic subsets of patients who could benefit from such a salvage chemotherapy.

PB1698

OMITTING CYTARABINE FROM CHEMOTHERAPY FOR ACUTE PROMYELOCYTIC LEUKEMIA REDUCES TOXICITY AND NOT EFFICACY

Background: The introduction of retinoic acid (ATRA) has changed the treat-
ment paradigm in Acute Promyelocytic Leukemia (APL). Combination of ATRA plus a consolidation chemotherapy (CO) is the standard of care worldwide. 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 con-
secutive treatment protocols that differed in AraC administration during con-
solidation.

Methods: We studied clinical characteristics, prognostic factors, response to treatment, toxicity, tolerance and outcomes in APL patients treated in our Department during the last decade. All patients received induction with AIDA (laronidase, ATRA until day 28), and 21 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 (PETHERA LPA2005).

Results: APL was diagnosed in 35 patients, of whom 2 patients older than 80 years did not receive treatment and were not included in the analysis. The rest 18 male: 15 female patients aged 37 (10-75) years old presented at diagnosis with: thrombocytopenia (32), leukopenia (22), leukocytosis (6), impaired per-
formance 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 com-
pared 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 [16(5-19) versus 9(0-18) days, p=0.002]. The same was true for neutropenia (p=0.04) and resulted to higher infection rates in Protocol 1 (58% versus 17%, p=0.03), including 2 aspergilloses 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 subse-
quent treatment decisions made for patients <60 and ≥60 years of age at the point of AML diagnosis and further investigated subsequent treatments.

Aims: This analysis examined the characteristics of patients <60 years of age and ≥60 years of age at the point of AML diagnosis and further investigated subsequent treatments.

Methods: Data from the Adelphi AML Disease Specific Programme, a real-
world, cross-sectional survey conducted between February–May 2015, were analyzed. A total of 61 hematologist/oncologists provided data on their 457 AML patients treated at various stages of AML. Disease characteristics upon
initial AML diagnosis including symptoms, performance status, and physician-determined prognostic category were taken from physician-completed patient record forms. Details about subsequently prescribed AML treatment were also taken from this data source. Treatments for n=15 (3.3%) patients were reassigned as high or low intensity following evaluation of physician treatment selection. Post-hoc T-tests and Chi-Squared/Fisher’s exact tests were used to determine differences between groups.

Results: Table 1 shows key presenting characteristics of AML patients <60 and ≥60 years old. According to physicians, those patients <60 years of age were significantly more likely than those ≥60 years of age to have de novo AML, a performance score of 0 versus ≤1 at diagnosis, more tests conducted to establish the diagnosis and a more favorable prognosis at baseline, according to physician perception. Following initial diagnosis, patients <60 years of age were 1.65 times more likely than patients ≥60 years of age to be initiated on high-intensity induction treatment: 67% (n=133) of patients <60 years of age versus 50% (n=98) of patients ≥60 years of age (p=0.012). Among those patients who had leukocytosis rate less than 20×10⁹/L and didn’t have CD56 and CD2 (11.5%) at the time of verification. The presence of FLT3 mutation was combined with high leukocytosis of the patient and with the absence of effect on the conducted therapy. When analyzing the immunophenotypic markers CD56 and CD2, they were detected in 75% of the patients, but in the absence of gene mutations and leukocytosis, such patients had a favorable prognosis (16.7% (13/18, p=0.049)).

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.

Table 1. Disease characteristics of patients <60 and ≥60 years of age at diagnosis of AML.

<table>
<thead>
<tr>
<th>Disease characteristic</th>
<th>&lt;60 years old (n=164)</th>
<th>≥60 years old (n=46)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>126 (77%)</td>
<td>17 (37%)</td>
</tr>
<tr>
<td>Pathophysiology</td>
<td>All four</td>
<td>204 (99%)</td>
<td>104 (88%)</td>
</tr>
<tr>
<td>Symptoms</td>
<td>No symptoms at diagnosis</td>
<td>30 (3%)</td>
<td>34 (29%)</td>
</tr>
<tr>
<td>Performance status</td>
<td>Normal</td>
<td>41 (38%)</td>
<td>41 (38%)</td>
</tr>
<tr>
<td>Diagnostic tests</td>
<td>No of tests used to establish AML diagnosis</td>
<td>5.5 (3.6)</td>
<td>4.7 (3.6)</td>
</tr>
<tr>
<td>Physician-determined prognosis category</td>
<td>Favorable</td>
<td>124 (75%)</td>
<td>24 (22%)</td>
</tr>
<tr>
<td>Intensive</td>
<td>Intermediate</td>
<td>84 (51%)</td>
<td>19 (16%)</td>
</tr>
<tr>
<td>Poor</td>
<td>Not determined</td>
<td>22 (13%)</td>
<td>39 (35%)</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 trend 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.

PB1700

FLT3, NPM1, CEBPA AND TP53 MUTATIONS AT ACUTE PROMYELOCYTIC LEUKEMIA: PROGNOSTIC FACTORS AND CORRELATION WITH OTHER MARKERS WITHIN THE PATIENTS OF GOMEL REGION IN BELARUS

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Background: Acute Promyelocytic Leukemia (APL) is one of the favourable variants of acute myeloid leukemias due to the usage of ATRA in the treatment similar to chemotherapy. But relapses occur in 13–33% cases after achievement the remission and there are cases of early death from the bleeding. High leukocytosis, the presence of lymphoid immunophenotypic markers and gene mutations are important prognostic factors.

Aims: To examine prognostic factors in APL.

Methods: The materials for research were the samples of whole venous blood and bone marrow of 40 patients with APL treated in the period of 2009-2016 in Hematology department for adults, Gomel. The diagnosis was proved by the presence (t(15;17)) or PML/RARA. Induction therapy was carried out according to the protocol t(15;17) using ATRA. Immunophenotypic analysis was carried out by standard immunofluorescence methods. The method of polymerase chain reaction (PCR) with specific primer and following electrophoretic detection was used for recognition of gene mutations.

Results: Out of 40 examined patients (mean age 48.5), 80% (32) achieved remission and 15.6% (5) subsequently relapsed after the first course of chemotherapy. Clinical, laboratory, molecular genetic and immunophenotypic data which could affect remission results and general survival rate were analyzed within all the patients. As a result, mutations were detected in 55% of cases, FLT3-ITD mutations were detected in 32.5%(13), NPM1 mutations in 12.5%(5), TP53 and CEBPA mutations were detected in 5%(2) and 12.5%(5) of cases respectively. After achievement of remission after the first course of chemotherapy NPM1 mutation remained at 6.2%(2). Mutations were identified more frequently within the patients with the absence of response to the therapy or with the developed relapse. The presence of leucocytosis was detected in 25% of cases, in 90% (9/10) of cases leukocytosis was combined with FLT3-ITD mutations and 80% of these patients subsequently had the recurrence. Within the patients with the combination of FLT3-ITD and NPM1 mutations who brought into remission after the first course of chemotherapy these mutations were not detected later on. There were the patients who had leukocytosis rate less than 20×10⁹/L and didn’t have CD56 and CD2 (11.5%) at the time of verification. The presence of TP53 mutation was combined with high leukocytosis of the patient and with the absence of effect on the conducted therapy. When analyzing the immunophenotypic markers CD56 and CD2, they were detected in 75% of the patients, but in the absence of gene mutations and leukocytosis, such patients had a favorable prognosis (16.7% (13/18, p=0.049)).

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.

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, hyperlipidaemia (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 reveal 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 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 ultrasound. 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 anticoagulant, anticardiolipin and antiphospholipid antibodies, anti-beta-2 glycoprotein-1, protein C-S, Antithrombin III and homocysteine levels, methylenetetrahydrofolate reductase, Factor V Leiden and prothrombin gene mutations no cause of tendency to thrombophilia could be determined. Echocardiography was normal. The patient was transferred to Cardiovascular Surgery Department for axillofemoral bypass operation.

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+hemmeraprex+ 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 aorticullosive 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 increasingly owing to the prolonged survival of patients treated with chemotherapy, tAL with mixed phenotype is extremely rare.

Aims: Here, we report a rare case of tAL with mixed phenotype with BCR-ABL1 after achieving complete remission (CR) of Diffuse Large B-Cell Lymphoma (DLBCL).

Methods: A 57-year-old woman was diagnosed as DLBCL. The patient received six cycles of R-CHOP regimen with G-CSF injected after each cycle and achieved CR. The patient was readmitted to the hospital after a follow-up examination revealed the presence of immature cells in the blood.

Results: Her complete blood count findings were as follows: hematocrit, 35.1%; hemoglobin, 116 g/l; platelet count, 129×10^9/L; and white blood cell count, 2.41×10^9/L, with 4% blasts, 26% segmented neutrophils, 3% band neutrophils, 39% lymphocytes, and 26% monocytes. Bone marrow aspirations revealed 40.7% blasts with medium cell size, oval-shaped vesicular nuclei, fine chromatin patterns, and basophilic cytoplasm. On cytochemical staining, these blasts were negative for 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 monocyte markers (cytoplasmic CD3 87%; CD5 90%; CD7 96%; cytoplasmic myeloperoxidase, 20%; CD 13, 91%; CD 33, 87%) and negative for CD2, CD10, CD11b, CD14, CD15, CD19, CD20, CD61, CD117, and TDT. Immunophenotype further filled the diagnostic criteria of T/myeloid biphenotypic leukaemia based on the scoring system of the EGIL and WHO classifications. Multiplex reverse transcription PCR using Human Leukemia (Ligand) (249) revealed presence of minor BCR-ABL1 (e1a2) fusion transcripts. Chromosome analysis of bone marrow cells failed because of insufficient mitotic cells. Immunoglobulin heavy chain gene rearrangement and TCR gene rearrangement were not detected on bone marrow aspirate.

Summary/Conclusions: Mixed phenotype acute leukemia is an uncommon subtype that comprises 0.5-1% of leukemia. The T/myeloid phenotype is rare 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 WHO 2016 WHO classification, tAL can be attributed to radiation, alkylating agents, or topoisomerase II inhibitors. Our patient did not receive radiation therapy but previously received cyclophosphamide and doxorubicin. Therefore, this is the first case of tAL with mixed phenotype and BCR-ABL1 after alkylating agent and topoisomerase II inhibitor therapy for DLBCL.

PB1704

CLINICAL, CYTOMORPHOLOGIC AND IMMUNOPHENO/IMMUNOHISTOCHEMICAL CHARACTERISTICS OF PATIENTS WITH BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASM - DIAGNOSTIC AND THERAPEUTIC DILEMMA

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Background: Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a clinically aggressive haematological malignancy that originates from clonal proliferation of plasmacytoid dendritic cells and their precursors. BPDCN is rare, represents less than 1% of acute leukemias. The disease has two patterns of presentations: cutaneous and leukemic. The main histological differential diagnosis includes: cutaneous NK/T-cell lymphoma; cutaneous T-cell lymphoma with co-expression of CD56 and CD56+ acute myeloid leukemia with monocydic differentiation.

Aims: The aim of study was to analyze heterogeneity of BPDCN differential diagnosis, especially with regards to clinical, immunological and cytological 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 35 years, range 26-60 years). Disease duration (average M/F: 12.6/14.2 months); weight (M/F: 67/68 kg); in the blood count, average concentration of Hb was 108g/l (range 87-154); WBC 6,38x10e9/l (range 2,6-12); Plt 147,8x10e9/l (range 20-282). Hemorrhagic diathesis was registered in 3/8; splenomegaly in 6/8 (average diameter by ultrasound exam 140mm, 110-150mm); and hepatomegaly existed in 3/8 pts (average diameter 166mm, 140-200mm). Cutaneous infiltrations were present in 5/8 pts as livid maculopapular rash along lower extremities in 5 pts, and in 1 female pts in the breast region of 1-4cm diameter. In all 5 pts, immunochemistry confirmed BPDCN diagnosis. In the bone marrow aspirates of 7/8 pts, average 75% infiltration (27-89%) with blasts was revealed. Cells were of median size, with high nucleus cytoplasmic ratio, with visible oval or slightly imprinted nuclei. Basic immunophenotype profile was characterized by expression of CD56+CD4+CD123+~high CD45RA ~, and negativity for CMPO- cCD79a - cCD3c- in 4 cases. Immunohistochemistry staining in the rest of 4 pts, characterized with dry aspiration, revealed LCA+CD4+CD56+CD4- CD33+ positivity and MPO- TDI-CD34 -CD117- CD68- HLA-DR- In 5/8 pts 1 pts had CD54 negative. Only 1 pts had CD54 negative. Cytochemical analysis revealed normal karyotype in 4 pts, while the rest of 4 pts had pathological findings: 1. 92.XX, XY; 2. 80-120,XXY,XY; 3. 16(16)X,XY,XY; 4. 46XY,del 5q/46XY,del 5q/46XY,del 5q, respectively.

Background: Translocation and chromosomal anomalies have prognostic implications in acute myeloid leukemia (AML). Cytogenetic analysis assumes great importance in their diagnosis and treatment stratification which are
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 letal outcome. One of the pts died within first 0,5 months after BPDCN was diagnosed. Three pts, treated with Hyper-CVAD, are alive and in CR with duration of 1, 3 and 10 months respectively. The continuation of the treatment within the programme of allogeneic stem cell transplantation is planned in 2 pts.

Summary/Conclusions: BPDCN diagnostics is difficult due to the heterogeneity of immunological characteristics of disease. Aggressive course of disease with median survival of 12-18 months, in the view of the unique treatment recommendations indicates necessity of further clinical investigations on larger patients groups.

PB1705

Aggressive Non-Hodgkin lymphoma - Clinical

ECONOMIC IMPACT AND HEALTHCARE UTILIZATION IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA IN ROUTINE CLINICAL CARE IN THE UNITED STATES – A CLAIMS DATABASE STUDY

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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: $7,486) 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 immunosorbent assay and assessed by a population pharmacokinetic analysis applying the non-linear mixed effects modelling.

Results: A 2-compartment model comprising linear non-specific clearance of 0.206 L/h (CI 0.207 – 0.279) L/day and inter-patient variability of 0.278 L (95% CI: 0.181 – 0.390) L/day, corresponding to target-mediated drug disposition of rituximab was recognised 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 0.82% lower (95% CI: 33.4 – 95.0).

Summary/Conclusions: These results imply that time-changes in clearance could serve as a predictive marker of response to rituximab. Our findings prove the rationale for studies evaluating higher doses of rituximab in selected patients.

PB1707

HOW 18FDG PET/TC CAN IDENTIFY BONE MARROW INFLTRATION IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA

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Background: Bone marrow infiltration (BMI) evaluation plays a key role in lymphoma staging, treatment and prognosis. The role of PET/TC in the assessment of BMI is still controversial, especially in non-Hodgkin’s lymphoma (NHL).

Aims: To evaluate the role of 18F-FDG PET/TC in bone marrow infiltration for the diagnosis of Non Hodgkin lymphoma. We compared 18F-FDG PET/TC visual and quantitative analyses with bone marrow biopsy in NHL patients.

Methods: Fifty patients with newly diagnosed NHL from February 2011 to February 2016 were retrospectively analyzed. Of these, 26 (group A) patients had aggressive NHL and 24 (group B) indolent NHL. To detect BMI on the posterior iliac crest bone marrow, two evaluation methods were used: 1) maximal standardized uptake values (SUVmax), cut-off >2.5, and 2) maximal standardized uptake values (SUVmax, cut-off >2.5), and 3) Deauville score (categorical). Each method was applied in the whole patients cohort, in group A and in group B. Images were blindly reviewed separately by 3 nuclear medicine physicians. PET/TC results were compared with the bone marrow biopsy performed after imaging in all patients. Positive biopsy bleeds was used to evaluate the increment in net benefit (NB) obtained considering the Deauville score over a biopsy-all strategy.

Results: The prevalence of a positive biopsy was 38% in whole cohort, 19% in group A and 58% in group B. In the whole cohort, sensitivity, specificity and accuracy were 60%, 82% and 67% for SUVmax, 58%, 85% and 68% for Deauville score. In group A, sensitivity, specificity and accuracy were 0%, 76% and 62%, for visual analysis; 40%, 52% and 50% for SUVmax; and 20%, 71% and 62% for Deauville score. In group B, sensitivity, specificity and accuracy were 29%, 100% and 58% for visual analysis; 64%, 60% and 62% for SUVmax; and 57%, 100% and 75% for Deauville score. At probability threshold equal to the prevalence of a positive biopsy, the increase in NB by Deauville score was 0.11 in the whole cohort, 0.02 in group A and 0.33 in group B. In this latter group, biopsying patients on the basis of the Deauville score is a strategy that reduced the biopsy rate by 24%, without missing any BMI.

Summary/Conclusions: FDG-PET/TC visual analysis has a limited value for detecting BMI in patients with NHL, while quantitative analysis by Deauville score provides a higher diagnostic performance. Noteworthy, the high positive predictive value in patients with indolent NHL suggests a potential role of FDG-PET/TC in avoiding bone marrow biopsy in this subtype of lymphoma.

PB1708

LOW ALBUMIN LEVEL CORRELATES WITH POORER SURVIVAL OF PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: SERBIAN LYMPHOMA GROUP EXPERIENCE

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Background: Current prognostic scores are not sufficient to define high risk patients with diffuse large B cell lymphoma (DLBCL). Besides parameters included in the International Prognostic Index (IPI), other clinical and laboratory parameters have been investigated as potential prognostic markers.

Aims: The aim of this study was to evaluate prognostic significance of clinical and laboratory parameters on the overall survival (OS) of patients with DLBCL.

Methods: A total of 393 patients (188 females/205 males) with the median age of 68 years (range 24-92) were included. All patients were initially treated with rituximab plus CHOP (Cyclophosphamide, Doxorubicin, 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 119 patients (30.4%). Following the Ann Arbor stage and the presence of bulky disease, effective prognostic factors were: Ann Arbor stage IV (p<0.0001), bulky disease (p<0.0001), low albumin level (p=0.002), platelet count less than 100x10⁹/l (p=0.0001), lactate dehydrogenase level over 250U/l (p=0.001), hemoglobin level less than 10g/dl (p=0.002) and involved sites involvement with lymph nodes (p=0.004).

Summary/Conclusions: Although BMI 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.
Methods: The Optum claims database was used to identify adult patients (≥18 years old) with newly diagnosed DLBCL between 01/01/08 and 10/31/15. DLBCL diagnosis was based on ≥1 inpatient claim or ≥2 outpatient claims with DLBCL diagnosis codes, with the index date being the first DLBCL claim. Patients were followed from index date until end of continuous enrollment, death, or end of study period (12/31/15). Treatment patterns and response to treatment were extracted from claims. Possible remission was defined as no additional chemotherapy and no supportive care use or receipt of supportive care <30 days after end of line of therapy (LOT) for <30 days. Lack of remission was defined as receipt of supportive care >30 days after end of LOT. Progression was defined as initiation of another LOT or evidence of supportive care >30 days after end of a LOT.

Results: Of the 2,216 patients selected into the study, 1,267 (57.2%) initiated 1LT, and median (interquartile range [IQR]) time to therapy was 0.7 (0.4–1.1) months. The majority of patients received combination (87.7%) vs single-agent (12.3%) chemotherapy. R-CHOP (60.5%) was the most frequently used combination. Chemotherapy in patients who achieved remission comprised 67% (8.2%) of single-agent use in 1LT. Median (IQR) duration of 1LT was 4.2 (2.3–4.5) months. At the end of 1LT, 64.0% (n=811) had evidence of remission, 15.0% (n=190) progressed, and 1.2% (n=15) had no evidence of remission. Second-line therapy (2LT) was initiated by 158 patients who progressed after 1LT; 29.6% received a single agent, and 70.4% received combination chemotherapy. In 2LT, rituximab (12.6%) remained the top single agent used, while bendamustine+rituximab (15.7%) and R-CHOP (8.2%) were the most common combinations; 82% of patients received stem cell transplant. Median (IQR) duration of 2LT was 2.1 (1.2–3.8) months. Of the 2LT patients, 44.0% (n=70) had evidence of remission, 26.4% (n=42) progressed, and 3.1% (n=5) had no evidence of remission. 34 patients who progressed after 2LT received third-line therapy (3LT); 29.4% received a single agent, while 70.6% received combination chemotherapy. In 3LT, rituximab (5.9%), etoposide (5.9%), and carboplatin (5.9%) were the most common single agents, while bendamustine+rituximab (20.8%) and etoposide+carboplatin+rituximab (17.6%) were the most common combinations; 8.8% of patients received stem cell transplant. Median (IQR) duration of 3LT was 3.5 (0.9–5.2) months. Following 3LT, 32.4% (n=11) had evidence of remission, 29.4% (n=10) progressed, and 5.9% (n=2) had no evidence of remission.

Summary/Conclusions: DLBCL treatment in routine clinical care aligns with guidelines; most patients receiving rituximab in combination with chemotherapy. A small proportion of patients received single-agent chemotherapy in 1LT. As expected, remission rates decreased with subsequent lines of therapy. Some patients were untreated; therefore, subsequent studies should explore reasons for lack of treatment.

PB1710

TP53 GENE MUTATIONS IS A PREDICTOR OF HIGH GRADE B-CELL LYMPHOMA PROGRESSION

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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 blockade 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 Ministry of Health Russian Federation diagnosis of high grade B-cell lymphoma were established in 47 patients: 13 – double hit, 34 – not otherwise specified. We had available biologic samples from 32 pts with HGBL: 11 – double-hit and 21 – NOS HGBL. 19 pts underwent courses of intensive treatment according to BL-M-04 [Efficacy and toxicity of a new short-term high intensity chemotherapy for primary malignant lymphoma; al. Haematologica. 2011; 96 (5): 391-392, 11 - R-(DA)-EPOCH, 2-R-CHOP-

Results: The correlation between MUT-TP53 and anti-tumor response in patients with HGBL is unclear.

Summary/Conclusions: Mutations in TP53 gene - a significant predictive factor of 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 neoplasm other than ATL.

Methods: We retrospectively analyzed 203 patients with HTLV-1 seropositive who were diagnosed between 2006 and 2015 at Kansai Medical University Hospital.

Results: Among 203 patients (median age 62 years: range 19 to 86 years), 43% was carrier and 57% was diagnosed with ATL. According to clinical subtype, 5% was chronic, 38% was smoldering, 28% was acute, 29% was lymphoma type. Median overall survival was 30 months in carrier, 10 months in acute, 8 months in lymphoma, and smoldering was not available. In all HTLV-1 seropositive patients, the occurrence of primary malignant neoplasm was 32%, they were all carrier or smoldering. Among them, 53% was hematology malignancy (T cell lymphoma; 41%, B cell lymphoma; 29%, MPN; 16%, MDS; 12%). Solid tumor was 47% (lung cancer; 33%, prostate cancer 13%, colon cancer; 13%, renal cell cancer; 13%). Four patients with HTLV-1 carrier who developed primary malignant neoplasm received standard chemotherapy for the neoplasm, and after the chemotherapy they developed 3 acute type and 1 smoldering type ATL.

Summary/Conclusions: In our cohort, the occurrence of primary malignant neoplasm with HTLV-1 seropositive patients was significantly high. Chronic HTLV-1 infection might associate with reduction of cytotoxic T cells and an increased risk of developing other malignancy. Furthermore, cytotoxic chemotherapy for primary malignant neoplasm might reduce cytotoxic T cells for HTLV-1 and exacerbate ATL conditions.

PB1712

Abstract withdrawn.

PB1713

THIOTEPA BUSULFAN CYCLOPHOSPHAMIDE, A TOXIC CONDITIONING FOR AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CENTRAL NERVOUS SYSTEM LYMPHOMA: REMISSION OR INFECTION

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Madrid, Spain, June 22 – 25, 2017

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Background: CNSL represents 4% of primary central nervous system (PCNSL) and secondary CNS lymphoma (SCNSL) occur in 7% of systemic lymphoma. Overall survival rates (OS) and progression free survival (PFS) have dramatically increased in PSNLCL since the introduction of Methotrexate high doses and ASCT usually conditioning with TBC (Thiotepa, Busulfan and Cyclophosphamide). The studies usually tend to recommend TBC/ASCT in front line for patients under 65 years with CNSL with very few prospective data about this strategy.

Aims: We report in this multicenter retrospective study our experience concerning TBC/ASCT and its main toxicities.

Methods: All patients treated with TBC/ASCT for PCNSL or SCNSL from August 2010 to November 2016 in our centers were researched by using CHIMIO® software. TBC combined Thiotepa (250mg/m²/d from d-9 to d-7), Busulfan (3.2mg/kg/d from d-6 to d-4), and 1.6mg/kg/d on d-4) and Cyclophosphamide (60mg/kg/d on d-3 and d-2) were followed by ASCT transplantation at d0. Clinical data were extracted from the medical records. We measured OS and PFS from the date of ASC and transplant related mortality (TRM) (defined by death occurred 3 months after ASCT).

Results: 24 patients, without any major co-morbidity, were included. Median age at ASC was 58 years (23-66). 22 of 24 were DLBCL and 2 follicular lymphoma and there were 15 PCNSL and 9 SCNSL. All but one, received 1 or 2 lines of chemotherapy (with high doses Methotrexate in first or second line) before ASC. 15 patients 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 in PR before ASC, 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 fungal 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 cho, 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 conditionings (for example TRM with Thiotepa Carmustine is increasing in PSNLCL since the introduction of Methotrexate high doses and ASCT usually conditioning with TBC (Thiotepa, Busulfan and Cyclophosphamide).

PB1714


Background: Using of Rituximab-containing regimens, as the «gold standard» strategy.

Aim of our study was to compare efficacy and toxicity of different treatment approaches in patients with diffuse large B-cell lymphoma (DLBCL). Shows significant improvement in the treatment results throughout all prognostic groups. The "real-life" treatment approaches vary depending on financial support of health-care system in different countries. Unfortunately, treatment results in patients with DLBCL from high and high-intermediate risk groups are still unsatisfying.

Methods: Prospective cohort study was initiated in 2014 in three Ukrainian centers. Patients with newly diagnosed or relapsed/refractory peripheral T cell lymphoma, not otherwise specified

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, D. Kato1, A. Tanaka2, N. Nakamura1, A. Fujimoto1, T. Yabushita1, Y. Shimomura1, Y. Ono2, A. Hashimoto2, N. Hiramoto3, S. Yoshioka1, N. Yonetani1, Y. Tanaka2, A. Matsushita1, H. Hashimoto2, S. Itoh3, T. Yabushita1

Background: The prognosis is extremely poor for cases of relapsed/refractory peripheral T cell lymphoma, not otherwise specified (PTCL-NOS), and there...
are no established predictors of prognosis. Although serum soluble interleukin-2 receptor (sIL-2R) levels are associated with clinical outcomes in newly diagnosed patients with PTCL-NOS, it remains unclear whether sIL-2R levels can predict prognosis in patients with relapsed/refractory PTCL-NOS.

Aims: This study evaluated whether sIL-2R levels at the time of salvage chemotherapy were associated with prognosis in cases of relapsed/refractory PTCL-NOS.

Methods: We retrospectively analyzed 45 patients with relapsed/refractory PTCL-NOS who received salvage chemotherapy at our institutions (1996–2016). All patients received CHOP or CHOP-like therapy as their initial treatment. The primary outcome was defined as overall survival (OS), which was calculated from the date of the salvage chemotherapy to the date of death from any cause or the last follow-up.

Results: The median age at salvage chemotherapy was 68 years (range: 37–86 years). The median serum sIL-2R level was 3,476 U/mL (range: 280–24,400 U/mL). Receiver operating characteristic curve analysis revealed that the optimal sIL-2R cut-off value for predicting OS was 2,283 U/mL (area under the curve: 0.672, 95% confidence interval [CI]: 0.421–0.923). Thus, we defined patients with serum sIL-2R levels of ≥2,283 U/mL as the high sIL-2R group and the other patients as the low sIL-2R group. The two groups had similar clinical characteristics at the salvage chemotherapy, with the exception of their international prognostic index (secondary IPI) and performance status (PS). The high sIL-2R group had significantly higher secondary IPI (≥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: 13.6–36.8), and the high sIL-2R group had significantly poorer 2-year OS (10.0%, 95% CI: 2.8–25.4 vs 50.0%, 95% CI: 24.5–71.0, P < 0.001). A multivariate analysis was performed using the following factors: serum sIL-2R levels (high vs low), secondary IPI (≥H-I vs ≤??????) (Figure 1).

Figure 1. Overall survival according to serum sIL-2R levels.

Summary/Conclusions: Serum sIL-2R levels are a useful predictor of prognosis in cases of relapsed/refractory PTCL-NOS, especially among patients with low secondary IPI risk.

PB1717

AUTOIMMUNE DISEASES ARE NOT ASSOCIATED WITH INFERIOR PROGNOSIS IN PATIENTS WITH LARGE B-CELL LYMPHOMA

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Background: Previous epidemiological studies have shown that autoimmune diseases increase the risk of lymphoma development. Immune dysregulation could be the possible underlying pathogenesis. Whether autoimmune diseases deteriorate outcome of lymphoma patients, however, remains unclear.

Aims: The objective of this study is to compare the clinical outcome among lymphoma patients with and without autoimmune diseases.

Methods: From January 2008 to November 2016, we retrospectively reviewed medical records of 913 newly diagnosed lymphoma patients. From these 913 lymphoma patients, 34 (3.71%) patients were diagnosed to have autoimmune diseases before their lymphoma identification. Among these 34 patients, six patients lost their 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 50.9±28.6 months, respectively (mean±standard deviation; p = 0.334). These two groups of patients had similar OS time as well (46.4±31.5 months vs 52.9±28.0; mean±standard deviation; p = 0.337). Univariate analysis did not show autoimmune diseases were associated with inferior OS in lymphoma patients (crude hazard ratio: 1.32; 95% confidence interval: 0.65–2.67, p = 0.023).

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/NON HEPATITIS DIFFUSE LARGE B-CELL LYMPHOMA EGYPTIAN PATIENTS

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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. Unlike all other malignancies, studies of the prognostic impact of miRNA-21 expression in diffuse large B-cell lymphoma (DLBCL) patients have been a matter of debate. To our knowledge, there are no existing data up to date on the expression of miRNA-21 in hepatitis C virus (HCV) associated DLBCL.

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. They were 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<0.001). Significant positive correlations between miRNA-21 and LDH, IPI and disease stage were detected (p<0.05). Significantly higher miRNA-21 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 prognosis (p=0.016), 2-year progression-free survival (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<0.001). 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 tumorgenesis, showing higher expression in HCV positive DLBCL patients compared to none hepatitis patients.
Background: Primary central nervous system lymphoma (PCNSL) is a rare type of non-Hodgkin’s lymphoma. Two independent prognostic scoring systems have been developed at the Memorial Sloan-Kettering Cancer Center (MSKCC) and the International Extranodal Lymphoma Study Group (IELSG). The former considers age and Karnofski’s performance status (PS) as prognostic parameters (JCO. 2006;24:5711). The latter includes age, Eastern Cooperative Oncology Group (ECOG) PS, the presence of deep lesions, serum lactate dehydrogenase (LDH) and total protein levels in the cerebrospinal fluid (CSF) (JCO 2005;23:2126). Neither of the two systems has been verified in the Asian population, leading to concerns regarding applicability in this region.

Aims: This study was conducted to test the prognostic power of the 2 systems in PCNSL patients in Taiwan. In addition, we analyzed the parameters of the IELSG system to figure out the most powerful prognostic factors and then established a new scoring system.

Methods: The medical records of patients with tissue-proven PCNSL were retrieved from 15 academic hospitals in Taiwan through January 2002 to December 2011. They were stratified into different groups according to the MSKCC or the IELSG system and the overall survivals (OS) were evaluated. All parameters in the IELSG system were checked by multi-variable analysis to establish a new scoring system.

Results: When the IELSG scoring system was applied, the 2-year OS in low, intermediate and high-risk groups were 78.3%, 43.9% and 37.5% respectively with a crossover in the latter 2 groups (Figure 1A). When the patients were stratified by the MSKCC scoring system, the 2-year OS of class I, II and III were 65%, 68% and 20% (Figure 1B), respectively. We conducted single-variable analysis of the 5 parameters included in the IELSG scoring system and only age and ECOG PS were statistically significant. In the multi-variable analysis, these 2 factors were almost equally weighted. Based on these findings, we re-stratified the patients into 3 groups. Group 1 comprised patients with both age <60 and ECOG PS <2 and Group 3 with both age ≥60 and ECOG PS ≥2. The patients not fulfilling criteria of either Group 1 or Group 3 were categorized as Group 2. According to this new scoring system, the median OS of Groups 1, 2 and 3 were 1,573, 548 and 304 days (Figure 1C), respectively, and their OS curves could be nicely distinguished.

Background: The incidence of lymphomas is increasing with age. Many aggressive lymphomas are now considered to be curable. All fit patients, even elders, are candidates for optimal treatment with a curative intent. Diffuse Large B Cell Lymphoma (DLBCL) is the most common non-Hodgkin Lymphoma, with 60% of curative rates after standard R-CHOP regime. Patients that relapse can be rescued with salvage treatment in 20-30%. The elders are not considered for full aggressive treatment in many centers. Geriatric scales are starting to be used to stratify patients and offer them individualized treatments. The use of GSCF for neutropenia prophylaxis is not a standard of care in this population.

Aims: The objectives of this study were: 1) Validate CIRS score in a DLBCL cohort; 2) Analyze the impact of CIRS score in OS; 3) Analyze the impact of GSCF 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 risk until population and evaluate the average of admissions stay and the impact on OS. The CIRS scale was adjusted by removing the hematological parameters in all our patients were diagnosed with a hematologic malignancy. The cut-off point for CIRS score was selected using a ROC analysis. Neutropenic fever (NF) events were recorded and the use of GSCF in prophylaxis were analyzed, as well as the admission days for adverse events.

Results: In our series, 20 patients (48%) were males. Median age at diagnosis was 73 years old (range 60-90) With a median follow-up of 32 mo. (range 0-96), the median PFS was 51 months and the OS was 61 mo. The patients were stratified by the R-IPI and the NCCN-IPI. The ROC analysis showed a scoring of 5.5 in CIRS to identify two different risk groups, with an AUC of 70.5%, a sensitivity of 87% and a specificity of 48% (p=0.02). In the low risk group, with CIRS ≤6 (n=17), 7 (41%) patients were admitted with a mean of stay of 6.2 days (range 1-62) vs the high-risk group with CIRS >6 (n=24). Of this group, 11(45%) patients were admitted with a mean of stay of 10.6 days (range 1-62), p=0.035. The CIRS scale was also used to discriminate two OS groups; the low risk showed a median OS not reached vs 29 mo. the high-risk group, with a Hazard ratio of 2.68 (CI95%; 1.031-5.882, p= 0.042). NF was the most common ER visit, n=18 (36%). Of the 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 prophylactic 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).

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 gives us a tool to help physicians to discriminate patients whom 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.
6/10 evaluated patients. Computed tomography scans showed slight to moderate contrast enhancement of adrenal masses in 4/5 patients (80%), and magnetic resonance imaging identified a normal T1 and longer T2 phase. Diffuse large B cell lymphoma (DLBCL) was the most common immunophenotype (82.6%). Two patients died due to rapid disease progression before treatment. Three patients were treated with chemotherapy/external beam radiation therapy. 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 adrenal mass with AI. Moreover, DLBCL and 51.0%, respectively. 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.

RESULTS: We investigated the clinical use of ibrutinib as a single-agent in 31 pts with relapsed or refractory MCL to obtain additional information about predictive factors, outcomes and toxicity in a real-life context.

Methods: We studied a group of 31 pts treated (or still in treatment) with ibrutinib to assess effectiveness in terms of overall response rate, complete response rate, progression free survival and adverse events (AEs) in a real-life context. Data were collected also with reference to clinical and biological characteristics of the disease (MIPI, MIPIb, bone marrow involvement, stage, histology, presence of bulky mass and/or extranodal disease) both at the time of diagnosis and at the time of the start of ibrutinib therapy, and to the type and number of previous therapies.

Results: Overall, 100% of pts treated with ibrutinib were assessable for effectiveness. The median age was 70 years (range, 45-82), 100% of pts had high risk MCL according to the MIPI score and 45.2% of pts presented extranodal involvement of MCL. 26 pts were treated for relapsed disease and one of them also presented 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 regimens. However, the quality and time of 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 alterations in blood pressure and lipid profile. Larger cohorts of pts and longer follow-up are warranted to confirm these preliminary data.

PB1723 HEMATOLOGICAL MALIGNancies IN SOLID ORGAN TRANSPLANT RECIPIENTS: RETROSPECTIVE SINGLE-CENTER ANALYSIS IN JAPAN K. Fujimoto1,*, I. Daiki2, R. Goto3, K. Morita2, T. Ooka4, K. Hatanaka5, H. Goto1, K. Hataou2, K. Hatanaka5, H. Goto1, K. Hataou2, K. Hatanaka5, H. Goto1, K. Hataou2, K. Hatanaka5, H. Goto1, K. Hataou2, K. Hatanaka5, H. Goto1, K. Hataou2, K. Hatanaka5, H. Goto1

Background: Solid organ transplant recipients have elevated onset risks of hematological malignancies (HMs) due to long-term administration of immunosuppressive agents. However, few studies about the incidence and impact on survival of HMs following solid organ transplantation have been conducted in Asian countries.

Aims: The aim of this study was to identify the incidence, characteristics, risk factors and prognosis of HMs in solid organ transplant recipients at our institution.

Methods: Clinical data of patients undergoing kidney, liver and heart transplantation at Hokkaido University hospital were prospectively collected. Fewer studies about the incidence and impact on survival of HMs following solid organ transplantation have been conducted in Asian countries.

Results: A total of 16 cases of HMs were identified, 9 post-transplant lymphoproliferative disorder (PTLD), 1 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), 0.9%, 2.6% at 5 years in liver transplant recipients (n=287) and 20% at 1 year heart transplant recipients (n=5), respectively (P<0.0001). AML/MDS and MPN were observed in 2.3% at 5 years and 20% at 10 years (P<0.01). There was no difference in background factors other than transplanted organ type between recipients with HMs and without HMs. Patients with EBV-positive PTLD (n=5) were younger (P<0.05) and had less extranodal diseases (P<0.05) compared with EBV-negative PTLD (n=4). All patients with monomorphic PTLD (n=4) were treated with chemotherapy combined with rituximab and had been in remission. In patients with other PTLD, reduction or withdrawal of immunosuppressant or rituximab alone resulted in stable disease or remission. All AML/MDS but 2 acute promyelocytic leukemia in pediatric patients were chemo-refractory and 19 pts were older than 60 years at diagnosis. Two patients received autologous stem cell transplantation as consolidation therapy. Five-year overall survival was 74%, 100% and 50% in patients without disease, with PTLD and with myeloid neoplasm, respectively. Survival in adult liver transplant recipients with myeloid neoplasms was inferior to that of non-Hodgkin lymphoma in general.

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 heightened awareness and better clinical approach for myeloid neoplasms following solid organ transplantation are needed.
variate analysis was performed for the OS. Elevated LDH level, stage ≥3, PS ≥2, ≥2 extranodal sites, IPI≥3, BCL6 negative (IHC), and MYC rearrangement (FISH) were significant factors in the female patients; however, PS ≥2 and IPI≥3 were significant factors in the male patients. Univariate analysis was also performed for PFS. Elevated LDH level, PS ≥2, IPI≥3, BCL6 negative (IHC), and MYC rearrangement (FISH) were significant factors in the female patients; however, PS ≥2 was the only significant factor in the male patients. Multivariate analyses were then performed using these factors in the Cox proportional hazard model. MYC rearrangement (FISH) [hazard ratio (HR): 9.13, 95% confidence interval (CI): 2.33–35.77, P=0.0015], and IPI ≥3 were identified as independent significant prognostic factor for OS in the female patients with DLBCL. Furthermore, MYC rearrangement (FISH) [HR: 2.47, 95% CI: 1.87–327.8, P=0.01494], and elevated LDH level were identified as independent significant prognostic factor for PFS in the female patients with DLBCL. On the other hand, PS ≥2 was identified as the only significant prognostic factor for OS (HR: 44.27, 95% CI: 6.71–292.2, P<0.001), but not for PFS in the male patients with DLBCL. Five of the 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).

Results: Cases with DLBCL of the stomach were excluded.

Methods: A retrospective single center study, including all consecutive DLBCL patients partial response and 6 patients were refractory. At last follow up, 13/18 patients achieved complete remission, 3 patients received anthracyclines containing chemotherapy, 3 cidofovir treatment and 2 palliative care. No stem cell transplantation was performed in any patient as a consolidation therapy. Four out of 13 patients achieved complete remission, 3 patients partial response and 6 patients were refractory. At last follow up, 13/18 patients were dead (11 lymphoma progression; 2 infectious complications). Median follow-up 63 months. OS in DHL/THL was 9 months and in DLBCL-noDH/THL was not reached (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/THL was 6 months (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/THL was 6 months (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/THL was 6 months (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/THL was 6 months (p=0.001). The PFS in DHL/THL and in DLBCL-noDH/THL was 6 months (p=0.001).

Background: Five to 15% of patients with diffuse large B cell lymphoma (DLBCL) present MYC and BCL2 and/or BCL6 rearrangements which are detected by fluorescence in situ hybridization (FISH) or standard cytogenetic. This rearrangement defines a subgroup of DLBCL so-called double hit or triple hit lymphomas (DHL/THL) which are included in the 2016 WHO classification revision of lymphoid neoplasms in a new category "High-grade B cell lymphoma with rearrangements of MYC and BCL2 and/or BCL6". DHL/THL have an aggressive clinical course and poor response to standard chemotherapy and a median overall survival of 0.2-1.5 years. The best therapeutic option in these patients is not yet well established.

Aims: To evaluate retrospectively the incidence, clinical-biological characteristics, type of treatment, overall survival (OS) and progression-free survival (PFS) of patients diagnosed with DHL/THL and to compare them with patients with DLBCL without double/triple-hit genotype (DLBCL-noDH/TH) in a single institution.

Methods: From January 2000 to April 2016, we analyzed 18 patients with DHL/THL and 312 patients with DLBCL-noDH/THL. DHL/THL cases were identified using FISH for MYC, BCL2 and BCL6 in the tumor tissue (11 lymph node biopsy, 2 gastrointestinal biopsy, 1 bone marrow biopsy, 3 skin biopsies and 1 cerebrospinal fluid).

Results: The incidence of DHL/THL was 5.5%. The median age was 70 years [range 53-93]. The patients included in DHL/THL group had a higher prevalence of advanced disease and higher IPI (p=0.002). Thirteen patients received anthracyclines containing chemotherapy, 3 cidofovir treatment and 2 palliative care. No stem cell transplantation was performed in any patient as a consolidation therapy. Four out of 13 patients achieved complete remission, 3 patients partial response and 6 patients were refractory. At last follow up, 13/18 patients were dead (11 lymphoma progression; 2 infectious complications). Median follow-up 63 months. OS in DHL/THL was 9 months and in DLBCL-noDH/THL was not reached (p<0.001). The PFS in DHL/THL and in DLBCL-noDH/THL was 5.4 and 63 months, respectively (p<0.001) (Figure 1).

Figure 1. Overall survival.
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 among of 30% of patients present a refractory/resisting disease following R-CHOP treatment. Rituximab-containing salvage chemotherapy followed by high-dose therapy and autologous stem cell transplant (ASCT) in chemosenstive patients remains the standard of care for these patients. Aims: We aimed to study the clinical features and outcome of patients diagnosed of DLBCL, homogeneously treated with R-CHOP/R-CHOP-like first line regimen, who have primary refractory disease (PRD).

Methods: Three hundred and sixty-seven patients were diagnosed of DLBCL between January 2004 to August 2016 in our center. 317 (86.3%) were treated with R-CHOP or R-CHOP-like in first line. Forty-four (13.9%) patients had PRD and PRD (12.3%) progressed during the follow up. Survival curves were estimated using the Kaplan-Meier method and compared using the Log-Rank test. Univariate analyses were performed by Chi square test and multivariate analyses by Cox proportional hazard regression model.

Results: Among the 44 primary refractory patients, 15 (34%), with a median age of 76 years (range 55-91), were considered unfit, 11 received supportive care and 4 were treated with palliative chemotherapy (cyclophosphamide and etoposide). 80% of patients underwent high dose chemotherapy according to FEAM regimen, who have primary refractory disease (PRD). 1 patient (10%) required a second collection procedure. Plerixafor was not used in any patient. The rates of successful consolidation with ASCT was 90%: CR 90% and PD 10% (1 patient). Overall, the rates of successful mobilization and effective as a CD34+ stem-cell mobilizing regimen. No data are available studies suggested that RBAC combination (with Cytarabine 800 mg/mq) is safe and effective as a CD34+ cells >= 2 x 10^6/Kg. The G-CSF (Lenograstim) infusion started per protocol at day 6 at dose of 5 umg/kg.

Results: All patients completed the scheduled treatment (4 cycles). The ORR was 80%. CR 90% and PD 10% (1 patient). Overall, the rates of successful mobilization and the proportion of patients achieving a total PBSC yield of >=2x10^6/kg were 100%, and the median PBSC yield was 10 x 10^6/kg (range 3-20x10^6/kg). The median time to stem cell harvest was 17 days (range 14-19). The median number of apheresis to achieve the PBSC target was 1 and only 1 patient (10%) required a second collection procedure. Plerixafor was not used. 80% of patients underwent high dose chemotherapy according to RBAC protocol (Filotemustine 150 mg/mq on days 1-4, Etoposide 200 mg/mq and Cytarabine 400 mg/mq on days 1-7) and achieved a CR. The median follow up of 43 months was 43 months and 90% were 80% respectively.

Summary/Conclusions: As in the relapsed/refractory setting and in MCL patients ineligible for high dose chemotherapy, RBAC has been proven to be an efficacious induction and mobilization regimen also in transplant eligible MCL patients with an encouraging safety profile. Further investigations are needed to assess the optimal role of RBAC within the standard first line treatments.

PB1728

RITUXUMAB BENDAMUSTINE CYTARABINE IS A FEASIBLE AND SAFE INDUCTION REGIMEN PRIOR TO ASCT IN FRONTLINE MCL: A SINGLE CENTER RETROSPECTIVE REAL LIFE EVALUATION

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Background: Mantle cell lymphoma (MCL) is an uncommon, still incurable subtype of non Hodgkin lymphoma. The routine use of high dose Cytabrine and high dose chemotherapy followed by autologous stem cell transplant (ASCT) markedly improved the outcome and has become the standard treatment for young (<65 years) patients. Recent studies demonstrated that Rituximab, Bendamustine and Cytabrine (RBAC) combination has a remarkable activity with a favorable safety profile both in untreated and relapsed/refractory elderly MCL patients (Visco et al., 2013 and 2017). These studies suggested RBAC combination (with Cytabrine 800 mg/mq) is safe and effective. Aim of this single center retrospective analysis was to assess the activity and safety of RBAC regimen in elderly MCL patients 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 etoposide). 80% of patients underwent high dose chemotherapy according to FEAM regimen, who have primary refractory disease (PRD). 1 patient (10%) required a second collection procedure. Plerixafor was not used in any patient. The rates of successful mobilization and effective as a CD34+ stem-cell mobilizing regimen. No data are available studies suggested that RBAC combination (with Cytarabine 800 mg/mq) is safe and effective as a CD34+ cells >= 2 x 10^6/Kg. The G-CSF (Lenograstim) infusion started per protocol at day 6 at dose of 5 umg/kg.

Results: All patients completed the scheduled treatment (4 cycles). The ORR was 80%. CR 90% and PD 10% (1 patient). Overall, the rates of successful mobilization and the proportion of patients achieving a total PBSC yield of >=2x10^6/kg were 100%, and the median PBSC yield was 10 x 10^6/kg (range 3-20x10^6/kg). The median time to stem cell harvest was 17 days (range 14-19). The median number of apheresis to achieve the PBSC target was 1 and only 1 patient (10%) required a second collection procedure. Plerixafor was not used. 80% of patients underwent high dose chemotherapy according to RBAC protocol (Filotemustine 150 mg/mq on days 1-4, Etoposide 200 mg/mq and Cytarabine 400 mg/mq on days 1-7) and achieved a CR. The median follow up of 43 months was 43 months and 90% were 80% respectively.

Summary/Conclusions: As in the relapsed/refractory setting and in MCL patients ineligible for high dose chemotherapy, RBAC has been proven to be an efficacious induction and mobilization regimen also in transplant eligible MCL patients with an encouraging safety profile. Further investigations are needed to assess the optimal role of RBAC within the standard first line treatments.
Aims: The objective of this retrospective, observational study is to evaluate the efficacy and safety of liposomal cytarabine in patients with CNS infiltration by haematological malignancies.

Methods: 36 consecutive patients with haematological disease and risk of CNS infiltration underwent flow cytometry (FC) analysis of CSF in a single center from December 2014 to December 2016. CNS involvement was assessed by using standard CSF cytology, 8-color flow cytometry or MRI imaging. Along with systemic therapy, all patients considered positive were treated 50 mg of IT Liposomal cytarabine administered by lumbar puncture every 2 weeks for 4 doses and every 4 weeks thereafter. Concomitant dexamethasone for arachnoiditis prophylaxis was added both i.v. and IT. We analysed the rate of adverse events (AE) and the time for 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 rejected systemic therapy as CSF analysis was not done due to co-morbidities in this age-group present significant challenges and complications resolved or improved with time.

Summary/Conclusions: Use of liposomal formulation of cytarabine for IT administration has become an effective option for the treatment of leptomeningeal involvement by haematological malignancies. Neurological AE are reversible; however, they accumulate and worsen with time, thus precluding long-term use.

PB1730

RETROSPECTIVE ANALYSIS OF OUTCOMES FOR ELDERLY PATIENTS WITH STAGE 3 AND 4 DISEASE HIGH-GRADE LBCL WITH REDUCED CYCLES OF R-CHOP OR R-GCVP: A 7 YEARS SINGLE-INSTITUTE EXPERIENCE.

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Background: The most common high-grade lymphoma malignancy in adults is Diffuse Large B-Cell Lymphoma (DLBCL), which has an increasing incidence with age (1). Over 40% of patients with DLBCL are above the age of 70, and the co-morbidities in this age-group present significant challenges and complexities with regards to selecting and implementing treatment regimens (2).

Aims: We present a retrospective analysis of outcomes for patients with high-grade DLBCL (stage 3 or 4 disease) who have received fewer than 6 cycles of full dose R-CHOP or R-GCVP because of poor tolerability or disease progression with treatment.

Patients and Methods: Retrospective data were collected from the cancer registry for all newly-diagnosed DLBCL patients who received R-CHOP or R-GCVP chemotherapy, with data collected from Jan 2010 to Feb 2017 from Ipswich Hospital 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 treatment. Secondary end points were overall response rate (ORR), overall survival (OS), and the reasons for premature ceasing of treatment based on graded toxicity according to NCIC-CTCAE 4.0.

Results: Of 87 patients, 12 patients were identified that fulfilled the inclusion criteria. The median age of patients was 72 years (range: 64-88 years), sex distribution was 7 male: 5 female, ECOG PS was 0-2 in 10 (83%) and ≤ 2 in 17% of the patients, Ann-Arbor Stage was 3 in 6 patients (50%) and 4 in 6 patients (50%), and IPI score was 3 in all 12 patients. 11 patients received R-CHOP and 1 patient received R-GCVP. The median length of treatment was 3.5 cycles (range: 1-6 months). The overall response rate was 50% at 50% of cycles 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 (n = 12) was 9.5 months (range: 2-42 months) and the median overall survival of living patients (8 out of 12) is at 40.5 months (range: 27-84 months). 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 co-morbidity. Further research on disease characteristics including molecular profile is needed to elucidate selected populations who may achieve long-term remission with shorter cycles of chemotherapy. Further insights may derive, for example, from analysis of polymorphism of folate pathway genes and/or of NF-kB, which have been previously suggested as pharmaco-genomic targets in lymphoid neoplasms. A risk stratification model needs to be developed to reduce drug toxicity and other short and long term treatment related complications so as to improve patient experience, and pharmaco-economic benefits.

PB1731

MULTIPLE NEOPLASMS CONSIST OF SOLID CANCER AND NON-HODGKIN LYMPHOMA

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Background: Malignant lymphoma is a ninth cause of death in Japan. And 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, the synchronous type is diagnosis into two different cancers in less than 6 months, metachronous type interval is more than 6 months. About statistical examination, we used SPSS statistics version 21.

Results: All cases are 176 cases, consist of male 108 cases, female 68 cases, synchronous type 45 cases, metachronous type 131 cases. Double neoplasms 149 cases, triple neoplasms 25 cases, quaduple neoplasms 2 cases. The median age was 7,7 years (ranged 51-88 years), the metachronous type was 73 years (ranged 57-93 years). The counterpart of malignancies, Hodgkin’s lymphoma 1 case, myelodysplastic syndrome 3 cases, acute myeloid leukemia 8 cases, multiple myeloma 4 cases, gastric cancer 6 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, uterin cancer 7 cases, esophageal cancer 9 cases, hepatocellular carcinoma 12 cases. In double neoplasms was 149 cases, metachronous type was 112 cases. The median age of first diagnosis, 68 years (ranged 43-85 years), the second cancer were 74 years (ranged 57-89 years). About interval between solid cancer and NHL, median interval time was 58 months, solid cancer precendence case was 53 cases, interval was 81 months (ranged 7-564 months), hematological malignancy precendence case was 59 cases interval was 55 months (ranged 8-364 months). 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 18 months (ranged 1-211 months), synchronous type 14 months (ranged 2-132 months), metachronous type 22 months (ranged 1-116 months).

Summary/Conclusions: In the case of a double cancer including solid cancer and NHL, the first cancer occurs in elderly. Diagnosis of malignant neoplasms (4 out of 149 cases(32.2%)). The important point is that 3 years are required for careful observation at the time of malignancy diagnosis. It is necessary to discover at the early stage. So it could be a lot of treatment options formalinagel 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 lymphomas
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 extra-nodal site.

Aims: Our aim was to define the efficacy and feasibility of this frontline strategy and eventually identify the subgroups of patients who may benefit from this approach.

Methods: We retrospectively analyzed 20 patients affected by aggressive DLBCL treated with R-CODOX-M/IVAC. R-CODOX-M consists of rituximab 375 mg/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, methotrexate 6700 mg/m². IVAC-R contains rituximab 375 mg/m², ifosfamide 1500 mg/m² day 1-5, etoposide 100 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 I and 5 in stage I. Twelve patients had B symptoms. Median IPI score was 3. Eleven patients had DPLs and 4 of them had TPLs. Overall survival (OS) was calculated from the time of diagnosis to the time of death or last follow-up.

Results: After a median follow-up of 28 months, 5 patients died (25%). OS at six and twelve months was 89.4% and 70.4%, respectively, median not reached (NR). Complete remission was achieved in 11 patients (59%), partial remission in 2 patients (10%). The overall response rate was 82%. Three patients (18%) were primary refractory. Among DPLs, OS was 83 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 had a better survival than obese patients, which is not statistically significant (p=0.77).

Summary/Conclusions: Obesity was associated with shorter survival among older patients with DLCLB treated with different chemotherapy regimens. The impact of gender on PFS and OS varied with BMI. The use of anthracyclin did not influence the outcome of obese patients. This study suggests that BMI may predict survival in older patients with newly diagnosed DLCLB.

PB1734

STOMACH DIFFUSE LARGE B-CELL LYMPHOMA: A SINGLE CENTER EXPERIENCE

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Background: Primary gastric diffuse large B cell lymphoma is a rare type of diffuse large B cell lymphoma. Immunochemotherapy followed by consolidation radiation is the conventional treatment. However, the cycles of chemotherapy and the role of consolidation radiation are still under debate.

Aims: To review and analyze the treatment experience of newly diagnosed primary gastric diffuse large B cell lymphoma. We presented the treatment outcome of our institution.

Methods: We retrospectively reviewed medical records from Jan 2005 to Dec 2014 from our institution. 30 patients with primary gastric diffuse large B cell lymphoma were included. Clinical characteristics, treatment regimens, treatment response, treatment modality, and survival were analyzed.

Results: From Jan 2005 to Dec 2014, there were 30 patients with primary gastric diffuse large B cell lymphoma. Median age was 65 years of age, 53% (n=16) of patients were male. All 30 patients (100%) have received chemotherapy. 13 of them (43%) have received involved field radiation therapy (IFRT). RCHO or RCEOP was administered in 86% (n=26) of patients. Complete response (CR) rate was 86% (n=24), 5-year survival was 69%. In patients who achieved complete response (CR), cycles of chemotherapy for 4 cycles of chemotherapy for 86% were vs 86% (p=0.42), respectively. For 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 resistance 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 disease with variable clinical course. 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.

Methods: We retrospectively analyzed 20 patients affected by aggressive DLBCL treated with different chemotherapy regimens. The impact of body mass index (BMI) on overall survival (OS) or progression-free survival (PFS) of diffuse large B cell lymphoma (DLCLB). Many factors like drug distribution and drug metabolism might influence the outcome in patients with excess body weight. The best method to predict outcome and adjust therapeutic approach is not known and elderly DLCLB patients do not receive appropriate therapeutic regimen.

Aims: To evaluate if BMI at diagnosis can predict clinical outcome in older patients with DLCLB receiving the first-line chemotherapy.

Results: After a median follow-up of 43 months (range, 1-128), median OS times were 19 months (4-not reached) for obese, 54 months (not reached) for underweight, 90 months (53-not reached) for normal weight and not reached for overweight. PFS was 18 months (4-not reached) for obese, 67 (51-not reached) and 91 months (53-not reached) for overweight and normal weight. In the treatment of non-antracyclins and overweight patients, the same chronological age, frequency was used antracyclin based regimen (CHOP, 27 patients; mCHOP, 22 patients; CVP, 7 patients; CEP, 7 patients). There was no difference in the frequency of different regimens in obesity group. In the group of patients treated with antracyclin based regimens, obese patients tended to have shorter survival, 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-antracyclins regimen treated patients, had obese patients, median OS 26 months (9-not reached). Overweight females and men with normal weight had a better survival than obese patients, which is not statistically significant (p=0.77).

Summary/Conclusions: Obesity was associated with shorter survival among older patients with DLCLB treated with different chemotherapy regimens. The impact of gender on PFS and OS varied with BMI. The use of anthracyclin did not influence the outcome of obese patients. This study suggests that BMI may predict survival in older patients with newly diagnosed DLCLB.
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, 7 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 those patients who received surgery as second line treatment some were classified as BCL2, and 1 MYC/BCL6. The average time to next treatment (TNT) was 5.2 months (0.5-19) for first line and 4.9 for third line. Mortality rate was 45.2%. With a median follow up of 18.6 months (3-58.6), the overall survival was 24.6 months (3-62).

Summary/Conclusions: The identification of biomarkers by immunohistochemistry is a relatively inexpensive process, which, when well elaborated and interpreted, allows to find in a safe way, subgroups of patients at high risk, who benefit from more aggressive 1st line therapy and, whenever possible, from the Inclusion in clinical trials with new drugs.

PB1736
INVESTIGATION ON TREATMENT STRATEGY, PROGNOSTIC FACTORS, AND RISK FACTORS FOR EARLY DEATH IN ELDERLY TAIWANESE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Given that the population of elderly cancer patients, including those with diffuse large B-cell lymphoma(DLBCL), is increasing, the management of cancer in the elderly has emerged as an increasingly common problem.

Aims: This study aimed to investigate the treatment strategy, prognostic factors, and risk factors of early death in elderly patients (age ≥65 years) with DLBCL in the rituximab era.

Methods: Elderly patients diagnosed with DLBCL between 2008 and 2014 were enrolled for analysis.

Results: There were 145 elderly patients with DLBCL diagnosed between 2008 and 2014. After excluding patients with primary central nervous system DLBCL (n=9) and incomplete data (n=3), a total of 133 patients (64 male and 69 female) with a median age of 74 years (range 65 to 94 years) were enrolled in the present study. Patients at a younger age and with better performance status were more likely to receive intensive frontline treatment. The median progression-free survival (PFS) and overall survival were 15 and 21 months, respectively. Anthracycline-containing chemotherapy achieved a higher remission rate and showed a trend toward better overall survival at the expense of a higher risk of severe neutropenia. Multivariate analysis revealed that very old age (≥81 years), a high-risk age-adjusted international prognostic index (aaIPI) score, and bone marrow involvement were associated with poorer PFS and overall survival. Progression of lymphoma was the major cause of death in the study population. In addition, approximately 25% of patients died within 120 days of their diagnosis. The risk factors for early mortality included very old age, a high-risk aaIPI score, and bone marrow involvement. The appearance of symptoms or signs of tumor lysis syndrome at diagnosis was associated with a trend toward early death.

Summary/Conclusions: Treatment of elderly patients with DLBCL remains a challenge, and comprehensive co-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 EPITHELIOEITROPIC INTESTINAL T-CELL LYMPHOMA: EXPERIENCE FROM AN ASIAN 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 (pathologically confirmed 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 CHOEP regimen, while another patient underwent surgery alone. The remaining two patients (1/4, 25%) had received surgery followed by chemotherapy (one with CHOEP, the other with BFM-90 protocol). Only one patient (1/4, 25%) entered complete response. Of concern, the unique patient achieved complete response received surgery followed by chemotherapy with Berlin-Frankfurt-Munster(BFM)-90 protocol. Remission duration was 10.3 months. He passed away 15.0 months after remission because of relapsed lymphoma.

Summary/Conclusions: Though the prognosis of MEITL is poor, operation followed by high dose chemotherapy such as BFM-90 protocol may have better treatment response, response duration and survival. It deserves further investigation.

PB1738
OSTEOPONTIN AS PROGNOSTIC FACTOR OF DIFFUSE LARGE B-CELL LYMPHOMA
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Background: Diffuse large B-cell lymphoma (DLBCL) is the most common type of lymphoma. It is a heterogeneous disease whose prognosis depends on the histological subtype (central germinal, non-centergerminal), as well as other factors such as age, clinical stage, extranodal disease, ECOG scale and levels of lactate dehydrogenase (LDH) identified by established scales (IPI, NCCN-IPI). Osteopontin (OPN) a protein that is secreted by various cells and fulfills physiological functions, when produced by neoplastic cells favors tumor growth and metastasis. This has been corroborated in different types of cancer and there are few reports of cases of patients with DLBCL in which the tumor expresses osteopontin, characteristically these cases have presented an aggressive clinical behavior with extranodal disease.

Aims: To evaluate the expression of osteopontin in neoplastic lymphocytes and their association with overall survival; the percentage of patients who expressed osteopontin at diagnosis; the association between the expression of osteopontin and the histological subtype (gumeral center, non-centergerminal, unclassifiable); the association between osteopontin expression and age, elevation of DHL, ECOG scale, clinical stage, extranodal invasion and the application of the IPI and NCCN-IPI scales.

Methods: Tissue samples were obtained from DLBCL patients diagnosed at the Instituto Nacional de Cancerología between 2014 and January 2016. Morphologic and immunohistochemistry features were studied on paraffin-embedded tissue microarray (TMA). Single antibody staining was performed for OPN. OPN expression was semiquantitatively assessed by three different pathologists scoring the proportion and intensity of stained cells. Positive cases were those that showed some degree of expression in the nucleus or cytoplasm of the tumor cell. Age, ECOG, clinical stage, LDH, extranodal invasion, histological subtype, IPI and NCCN-IPI score were independently documented. Overall survival (OS) analysis was performed by the Kaplan-Meier method, the comparison between different curves was performed using the log-rank test; for the analysis of the relationship between variables we used the χ2 test with a statistical significance of p<0.05.

Results: 81 patients were evaluable. 43.2% of the cases were positive for OPN in neoplastic cells. The mean survival of patients with positive OPN was 14.8 months versus 16.5 months for patients with no OPN expression (p=0.025). OPN positivity was not significantly associated with increased age, impaired functional status (ECOG 2.3,4), advanced clinical stage (III, IV), increased LDH or extranodal invasion (including central nervous system); neither was it associated with a specific histological subtype. Survival significantly decreased in patients with increased LDH (p=0.000137), ECOG 2.3,4 (p=0.0047). Survival decreased significantly as the risk measured by the IPI and NCCN-IPI scores increased (p=0.000001, p=0.000013 respectively) with an average survival of 18.6 months for the low-risk group, compared with 6.4 months for the high-risk group (Figure 1).
90 day mortality was 7 of all 3 groups is illustrated in Figure 1 below.

Figure 1.

Summary/Conclusions: Our findings demonstrate that approximately half of the cases evaluated express OPN at diagnosis and tend to have a lower survival rate; however, a longer follow-up time is needed, as well as other studies that discriminate between different isoforms or post-translational modifications of osteopontin to determine if this trend can reach significance. By demonstrating OPN expression by neoplastic cells we can devise new protocols that 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.

PB1739
TREATMENT OF NEWLY DIAGNOSED CENTRAL NERVOUS SYSTEM LYMPHOMA PATIENTS BASED ON COMORBIDITIES & PERFORMANCE STATUS: A SINGLE-CENTRE EXPERIENCE
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Background: Combination chemotherapy incorporating high dose methotrexate (HD-Mtx) and high dose cytarabine (Ara-C) is the standard chemotherapeutic approach for newly diagnosed primary CNS lymphoma (PCNSL). However, patients >60 years old account for 50% of cases and combining HD-Mtx with Ara-C may be associated with high toxicity and early mortality. The management of secondary CNS lymphoma (SCNSL) is less clear, but is often based upon a similar approach.

Aims: Present a tertiary centre experience in management of primary (PCNSL) and secondary CNS lymphoma (SCNSL), with therapy based on co-morbidities and performance status.

Methods: We performed a retrospective analysis of patients with a diagnosis of CNS lymphoma seen at our centre between 2011 and 2016. These were categorized into 3 groups, Group 1: treatment of newly diagnosed PCNSL prior to September 2014 where majority of patients received HD-Mtx & Ara-C combination chemotherapy, Group 2: treatment of PCNSL after September 2014 where patients were selected based on co-morbidities to receive Mtx with or without Ara-C, Group 3: treatment of newly diagnosed SCNSL. The median survival for each group was estimated using the Kaplan-Meier method and log-rank test. Overall response rates, 30 day and 90 day survival between groups 1 & 2 were compared using unpaired t test.

Results: 60 pts with a median age of 65 years old were recruited. 40 pts were diagnosed to have PCNSL at presentation, while 20 patients had SCNSL. 5 pts were excluded from this study as they did not receive any treatment. In group 1, 21 pts (84%) received combination chemotherapy incorporating HD-MTX and Ara-C; 3 pts (12%) received HD-MTX monotherapy and 1 pt (4%) received radiotherapy only. In group 2, 7 pts (53.8%) received HD-MTX and Ara-C as part of MATRIX protocol or with single agent rituximab, 3 pts (23%) received HD-MTX as part of RMP protocol or with single agent rituximab, 1 pt (7.7%) received a single alkylating agent and 1 pt (7.7%) received radiotherapy only. In group 3 15 pts (88.3%) received chemotherapy incorporating HD-MTX and Ara-C; 2 pt (11.8%) received HD-MTX without Ara-C. 30 day mortality was 7 (28%) in group 1 and 0 in group 2 (0%) (p=0.03), 90 day mortality was 7 (28%) in group 1 and 2 in group 2 (15.4%) (p=0.39). Overall response rate was 9 (36%) in group 1 and 8 (61.5%) in group 2 (p=0.13). A Kaplan Meier curve of all 3 groups is illustrated in Figure 1 below.

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.

PB1740
AN AUDIT OF THE USE OF RASBURICASE FOR THE PREVENTION AND TREATMENT OF TUMOUR LYMPHOSIDE IN PATIENTS RECEIVING TREATMENT AT THE NORTHERN CENTRE FOR CANCER CARE, NEWCASTLE UPON TYNE, UK
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Background: Tumour Lysis Syndrome (TLS) is a known complication of haematological oncological treatment. Although clinical TLS is rare, the consequences are significant, with one third of affected patients requiring dialysis and an overall mortality rate of around 15%. A new British Society for Haematology (BSH) guideline was published in April 2015 to guide physicians on how to risk stratify patients based upon the Cairo Risk Stratification 2010 criteria/BSH criteria as having High Risk Disease (HRD) or Intermediate Risk Disease(IRD)/Low Risk Disease (LRD)with renal impairment, and therefore should have received 3mg rasburicase prophylaxis if no evidence of TLS according to the guideline. Of those 20, 11 had laboratory TLS, and therefore BSH guidelines would recommend 0.2mg/kg/day [JG1] rasburicase, however only 3/11 were given the drug at treatment doses. 1/3 had clinical TLS at presentation and received treatment according to the guideline. The other 2 patients received larger doses of rasburicase but less than the BSH would recommend. A further 7 patients with IRD received rasburicase prophylaxis but on review did not meet the criteria for rasburicase as set out in the guidelines. 5 patients died during the study period. 2 patients died on ITU of multi-organ failure <7 days into chemotherapy. A third patient died of sepsis, and the other 2 deaths were in deteriorating patients where a decision was made to palliate.

Summary/Conclusions: When assessed against BSH standards, all patients in this cohort who should have received rasburicase prophylaxis, were given the drug. 2 patients with lab TLS received lower doses than the BSH would recommend, but did not progress to clinical TLS. Although there were 5 deaths in our cohort, none were directly attributable to TLS. In order to comply with the guidelines, particular importance must placed on formally assessing the TLS risk score as per Cairo
criterion at the outset and analyzing the possible features of laboratory TLS. Although dosing did not always follow BSH guidelines, we did respond to biochemical deterioration. The majority of patients with HD developed acute kidney injury despite rasburicase. Doses were increased in response to creatinine increases, albeit not as per guideline. It is noteworthy that despite lower than the recommended doses of rasburicase, 6/8 patients with lab TLS did not progress to clinical TLS and none required dialysis. The guideline is a good tool for the risk stratification and treatment of patients at risk of TLS. In clinical practice 100% compliance is hard to achieve. Responding to trends in creatinine may explain why, despite lower than recommended doses, our outcomes were still good. It would be interesting to see if further work with larger numbers of patients would support this. Since this audit was completed, the ePrescribing system has been altered to improve practice and a re-audit is planned.

PB1742

PROGNOSTIC IMPACT OF SYNCHRONOUS MULTIPLE PRIMARY MALIGNANT TUMORS ON NEWLY DIAGNOSED LYMPHOMA PATIENTS

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Background: Synchronous multiple primary malignant tumors (sMPMTs) are occasionally diagnosed during screening for a newly diagnosed malignant neoplasm. Lymphoma is one of the most common hematological malignancies, and number of lymphoma patients with sMPMTs seems to grow as the population ages. Since the standard chemotherapy for lymphoma takes a few months, treatment strategy sometimes comes to an issue.

Aims: We retrospectively analyzed patients with malignant lymphoma newly diagnosed between 2009 and 2015. The definition of sMPMTs was patients who were also diagnosed as a solid tumor within 6 months of the diagnosis of lymphoma. Therapeutic strategy was according to physician’s choice. Impact of sMPMTs on treatment outcome of lymphoma was analyzed. Also, relation between treatment of lymphoma and concomitant solid tumors was closely analyzed.

Results: Total of 505 lymphoma patients was included. Median age was 69 (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 the solid tumor. 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.

PB1741

IMPLICATION OF BASIC VALUES OF VITAMIN D IN THE CLINICAL COMPLICATIONS OF PATIENTS WITH NO HODGKIN LYMPHOMA IN ACTIVE CHEMOTHERAPY TREATMENT

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Background: The incidence and prevalence of Non-Hodgkin’s Lymphoma B (NHL-B) has increased in recent years, reaching approximately 3-7 cases / 100,000 habitants. For this reason, the number of patients who receive chemotherapy treatment is also considerably higher; this implies a greater presence of adverse events. In many of these patients, baseline vitamin D values at the time of diagnosis are decreased, and may be related to the development of the tumor pathology, also to the severity of the adverse events.

Aims: To assess the implication of vitamin D values in the development of relevant clinical complications in patients diagnosed with NHL-B receiving chemotherapy. To determine its clinical evolution after correcting the vitamin D deficit.

Methods: Retrospective study (January 2013-January 2017), which includes patients diagnosed with NHL-B with histological confirmation. We analyze demographic parameters (age, sex), histological subtype of NHL according to WHO classification, laboratory values of vitamin D (cut-off values: optimal 25-66 pg/mL; low 25-18 pg/mL or very low <18 pg/mL), adverse effects: hematological toxicity, infection, gastrointestinal toxicity, hospital admissions and exitus. A subanalysis of complications was performed in patients with vitamin D deficiency treated with vitamin D corrective treatment.

Results: 68 patients were analyzed, and 57 cases (84%) were valid because they had vitamin D determination in the 8 weeks near the diagnosis. The distribution were: 58% (n=31/54) with median age 59 years (range: 29-91 years). The subtypes of LNH-B: Follicular (23/40%), Diffuse large cell (21/37%), Mantle (6/11%), Marginal (4/7%) and others 3 (5%). Patients were included in 3 groups according to serum vitamin D levels: patients with optimal levels (n=23), low levels (n=27; 48%) and very low levels (n=7; 12%). Hematological toxicities were higher for the subgroup with decreased vitamin D levels vs subgroup with level in range (28% vs 72%) (p<0.05). Neutropenia was more severe (grade 4) in patients with very low levels of vitamin D (p<0.01). No patient with optimal vitamin D levels had severe anemia (Hb<8 g/dL) or thrombopenia (<70000/mm3), in relation to 28 cases of severe toxicity diagnosed in vitamin D deficient groups. In this group were documented the two infections of the study (both pneumonias), a gastrointestinal toxicity, and one exitus. After treatment, it was found that 74% (n=25 patients) corrected levels, presenting a lower incidence of toxicity to the treatment vs 26% (n=9) who did not correct levels and presented more complications (especially hematological toxicity) more complex and durable.

Summary/Conclusions: Vitamin D deficiency in the diagnosis of patients with NHL-B has been correlated with a higher incidence of medical complications due to the treatment of chemotherapy. In our series, patients had greater hematological toxicity and greater severity (p<0.01), more infectious episodes and a higher hospital admission rate. These adverse effects are even more pronounced in the level D levels <18 pg/mL. A study conducted by Drake et al. (JCO, 2010) on 980 patients presents similar data, with a significantly higher incidence of complications in vitamin D deficient patients. After treatment with vitamin D, patients who corrected levels had a more favorable evolution with fewer hematological and infectious complications (p<0.01) in relation to those patients in whom the vitamin deficit persisted despite the treatment. At this time, the monitoring period is not completed, so the data related to OS and SLE still have to be updated and will be presented at the next congress. We believe that the determination of vitamin D levels should be routinely included in the diagnosis in patients with NHL-B because could be a modifiable risk factor in the complications of this patients.
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 were subjected; 12 hemophilia A patients and 7 hemophilia B patients. Factor concentrates were injected to reach the target activity of 80% in hemophilia A and 40% in hemophilia B which is given by Korean health insurance guideline. All patients were in non-bleeding state and kept the wash-out period of 3 days 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 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 PAEDIATRIC HAEMOPHILIA CARE CENTRES

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Background: Development of neutralizing anti-factor VIII alloantibodies (inhibitor; INH) is the most challenging complication of haemophilia replacement therapy (HRT). It occurs in up to 30% of severe haemophilia A (HA) patients. Data published recently indicate that immunotolerance induction (ITI) is effective in 62–77% of cases.

Aims: To assess the rate of successful ITI in boys with severe HA treated with full length recombinant FVIII (octocog α) in all Polish Paediatric Haemophilia Care Centres between 2011-2016.

Methods: From 2011 to 2016 in all Polish Paediatric Haemophilia Care Centres between 2011-2016. The remaining 3 patients are still on ITI. All 7 patients after successful ITI were put back on prophylaxis with octocog α.

Summary/Conclusions: 1. Octocog α is effective in induction of immunotoler- ance in severe haemophilia A boys who developed inhibitor on prophylaxis with octocog α.

Table 1. Characteristic of patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>VWF:Ag</th>
<th>FVIII:C</th>
<th>FIX:C</th>
<th>INH:R</th>
<th>Pred. inhibitor dose</th>
<th>Number of EDs</th>
<th>Thromboelastogram (TEG)</th>
<th>Clot wave form analysis (CWA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>18</td>
<td>92</td>
<td>1</td>
<td>80</td>
<td>9</td>
<td>50</td>
<td>5</td>
<td>TEG index -2.6 ± 1</td>
<td>MIN2 -0.7 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>96</td>
<td>1</td>
<td>85</td>
<td>7</td>
<td>50</td>
<td>6</td>
<td>TEG index -2.4 ± 1</td>
<td>MIN2 -0.8 ± 0.2</td>
</tr>
<tr>
<td>C</td>
<td>12</td>
<td>98</td>
<td>1</td>
<td>88</td>
<td>5</td>
<td>50</td>
<td>7</td>
<td>TEG index -2.5 ± 1</td>
<td>MIN2 -0.8 ± 0.2</td>
</tr>
</tbody>
</table>

GO, on demand; P, prophylaxis; CVA, central venous access; N, no; V, yes; mth, month.

Background: Niemann Pick Disease type A and B is a rare autosomal recessive disorder caused by sphingomyelinas deficiency resulting in sphingomyelin accumulation in macrophages of varies organs. In type B usually patients survive in adulthood. Usually, they have hepatosplenomegaly, thrombocytopenia, and dyslipidimia. Liver and lter function are influent, and they have arthralgia, ataxia.

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 Niem- manick disease type B. She had marked splenomegaly, mild thrombocytope- nia 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.

Results: 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. Hyperlipoproteinemia type IIb with hypoHDL cholesterolemia was present. We assumed that platelets dysfunction could exist, therefore before planned amniocentesis we performed plateletless transfusions 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 sistem revielad liver diameter 17cm, cranio-caudal diameter of spleen 22cm, portal vein had not been seen. There were no sign of thrombosis in portal branches. Amniocentesis was done without complication 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 35th+5d gestational week she was oper- ated. Before surgery platelets count was 87x109/l, she was given seven concent- rates of platelets (1 per 10 kg body weight) before and seven during proce- dure. She also received antibiotic prophylaxis. Newborn was 47cm, 2490 weight and Apgar score was 7/8. There was no major blood loss and no need for red blood cell transfusion or platelets transfusion in follow up period. We decided not to make splenectomy or partial resection because there were no significant differences in spleen measurements before and during the preg- nancy, and there was no sign of spleen trauma. Also, in literature we found data about worsening lung function after this procedure caused by more shin- gomyelin accumulation in pulmonal tissue. Published data and findings of 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 find- ings of Niemann Pick disease in newborn.

Summary/Conclusions: We presented a case of pregnancy in 34 year old women with Niemann Pick disease type B. Marked splenomegaly, mild thrombocytopenia and partial respiratory insufficiency existed before this pregnancy. Decisions about diagnostic assessement, platelet transfusion, splenectomy, and
PB1746

SINGLE CENTRE FX DEFICIENCY EXPERIENCE
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ulty, istanbul, Turkey

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 hemorrhagic symptoms, including easy bruising, haematuria, soft-tissue hemorrhages, 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 a 40 years old man who got his diagnosis at the age of 31 years following a gastrointestinal bleeding. He was treated with fresh frozen plasma (FFP) at that time. His FX was found: 5%. Two years later underwent a planned tooth operation under the coverage of prothrombin complex concentrate (PCC) (Table 1). Three years after the tooth extraction he underwent an intraocular lens operation under PCC prophylaxis. No complication was observed while on PCC treatment.

Table 1.

<table>
<thead>
<tr>
<th>weight: 70 kg</th>
<th>PCC</th>
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<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 bleedings is 15-20 times in a year and most of them are gum bleeding and rarely vaginal bleeding. Third and 4th Patients were referred to our centre because of prolonged the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) and received the diagnosis of FX deficiency.

Summary/Conclusions: Bleeding phenotype differs in a wide range in patients with congenital FX deficiency. Secondary causes including amyloidosis should be excluded especially in patients receiving diagnosis at advanced ages. Usually the factor level does not correspond to the severity of the bleeding phenotype. Therefore bleeding pattern of the patients with FX deficiency should be carefully observed and considered while planning a prophylactic treatment with PCCs to prevent the risk for thrombosis and unnecessary utilisation of PCCs. FFP and PCCs replacement continue to be the source for FX in bleeding patients or in individuals requiring prophylaxis. Recently, a FX concentrate has entered the market in the USA and the European Community.

PB1747

IMPROVEMENT OF THE SURVIVAL FOR LIFE-THREATENING HEMORRHAGE WITH HEMOPHILIA PATIENT
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1Division of Pediatric Hematology, 2Department of Pediatrics, Dr. Behçet Uz Children's Hospital. Mothers whose at least one of the other members of the family and their sons had hemophilia, mothers with more than one hemophilic son and girls whose father had hemophilia were evaluated as an obligate carrier. Sisters or mothers who do not meet the obligatory carrier criteria but whose siblings or sons are hemophilic were identified as possible carriers. Factor activity of obligate or probable carriers was studied after their informed consent was obtained.

Results: Thirty-two mothers and 13 sisters of hemophilia patients were included in this study. The mean age was 31.6 (4-57) years. Three of the patients were mild, 3 were moderate, 23 were severe hemophilia A; 2 were severe and 1 had moderate hemophilia B. Twelve were obligate and 33 were probable carriers. Only seven in 45 (15.5%) probable and obligate hemophilia carriers had high bleeding scores (≥24). Those with high bleeding scores, three were obligate carriers and four were probable carriers. The mean factor activity of 12 obligate and 18 probable carriers were 78.9% (20.8%≥189%). Factor activities of the three obligate carriers with high bleeding scores were 77%, 80% and 98%, respectively. Factor activities of the three probable carriers with high bleeding scores were 58.8%, 69.3% and 112%, respectively. The median bleeding scores of four probable and one obligate carriers with low factor activity (<60%) were 2.8 (1-4).

Summary/Conclusions: Measurement of factor activity seems to be insufficient to detect hemophilia carriers. ISTH/SSC-BAT may help to determine the carriers. However, a larger study is needed to understand the diagnostic value of the BAT.

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 Behcet Uz Children’s Diseases and Surgery Training and Research Hospital. Mothers whose at least one of the other members of the family and their sons had hemophilia, mothers with more than one hemophilic son and girls whose father had hemophilia were evaluated as an obligate carrier. Sisters or mothers who do not meet the obligatory carrier criteria but whose siblings or sons are hemophilic were identified as possible carriers. Factor activity of obligate or probable carriers was studied after their informed consent was obtained.

Results: Thirty-two mothers and 13 sisters of hemophilia patients were included in this study. The mean age was 31.6 (4-57) years. Three of the patients were mild, 3 were moderate, 23 were severe hemophilia A; 2 were severe and 1 had moderate hemophilia B. Twelve were obligate and 33 were probable carriers. Only seven in 45 (15.5%) probable and obligate hemophilia carriers had high bleeding scores (≥24). Those with high bleeding scores, three were obligate carriers and four were probable carriers. The mean factor activity of 12 obligate and 18 probable carriers were 78.9% (20.8%≥189%). Factor activities of the three obligate carriers with high bleeding scores were 77%, 80% and 98%, respectively. Factor activities of the three probable carriers with high bleeding scores were 58.8%, 69.3% and 112%, respectively. The median bleeding scores of four probable and one obligate carriers with low factor activity (<60%) were 2.8 (1-4).

Summary/Conclusions: Measurement of factor activity seems to be insufficient to detect hemophilia carriers. ISTH/SSC-BAT may help to determine the carriers. However, a larger study is needed to understand the diagnostic value of the BAT.

PB1749

FETAL INTRACRANIAL HEMORRHAGE AS A PRESENTING FEATURE OF SEVERE CONGENITAL FACTOR VII DEFICIENCY: THE NEED FOR EARLY PROPHYLAXIS
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Background: Congenital factor VII (FVII) deficiency is a rare autosomal reca-

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sive bleeding disorder, with an estimated prevalence of 1:300,000. Compared to western countries, rare bleeding disorders (RBDs) are relatively common in Oman, owing to high rate of consanguineous marriage.

**Aims:** To discuss an interesting case of severe congenital factor VII deficiency and to explore the need for early prophylaxis.

**Methods:** Case report and retrospective data analysis of all children diagnosed with inherited coagulation factor deficiencies in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2009 till December 2016.

**Results:** We report a male full term baby, delivered by cesarean section. His older sister is a known case of severe congenital factor VII deficiency. Antenatal scans of this baby revealed two intracerebral hematomas and dilated cerebral ventricles. Postnatally, the diagnosis of severe congenital FVII deficiency was confirmed. CT scan revealed obstructive hydrocephalus at the level of aqueduct of Sylvius (Figure 1). At day 10 of life, ventriculo-peritoneal shunt has been done successfully under cover of recombinant activated factor VII replacement therapy. Afterwards, the patient has been initiated on rFVIIa prophylaxis at a dose of 30 ug/kg three times weekly. In our center, deficiencies of fibrinogen, FV, FVII, FX and FXIII were diagnosed in 22 pediatric patients (10 males and 12 females), accounting for 11.1% (22/198) of all children with inherited coagulation factor deficiencies. The age ranges from 1 day to 6 years and consanguinity is found in 19/22 cases (86.4%). Hypofibrinogenemia, FV and FVII deficiency are the commonest RBDs, diagnosed in 8, 6 and 5 patients respectively. As an initial presentation, intracranial hemorrhage occurred in 7/22 cases (31.8%). Three patients with FV, FVII and FXIII deficiencies suffered from global developmental delay due to severe intracranial hemorrhage. As regards management, 4 patients with severe FV deficiency and one with severe FXIII deficiency are on fresh frozen plasma (FFP) and recombinant FXIII prophylaxis respectively. Other patients receive on-demand therapy.

**Figure 1.**

**Summary/Conclusions:** Children with RBDs constitute more than one tenth of cases of hereditary coagulation factor deficiencies in our center. They have some unique features in terms of severity, clinical profile and the need for prophylaxis early in life. We recommend establishing a national/regional registry of RBDs to identify the magnitude and the peculiar genotype-phenotype correlations of such rare, yet significant disorders.

**PB1750**

**THE ASSOCIATION OF BLOOD TYPE WITH THE NEED FOR TRANSFUSIONS IN PATIENTS WITH VENTRICULAR ASSIST DEVICES**

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**Background:** Patients who have implantation of continuous flow ventricular assist devices (VAD) as a bridge to heart transplantation are subjected to complications secondary to pump support. The use of antiplatelets either alone or in combination with anticoagulation is necessary to avoid clot formation and pump thrombosis. However, a proportion of patients reveal an increasing risk of bleeding episodes. A possible reason of this situation could be that high shear forces lead to devastation of high molecular weight von Willebrand factor (vWF) making it functionally inactive and resulting in acquired von Willebrand disease (vWD). People with blood type O have lower baseline vWF levels and this abnormality could exacerbate the bleeding risk of patients with blood type O with VAD, resulting in more frequent bleeding episodes and need for transfusions.

**Aims:** The aim of current study was to investigate the possible association of blood type with acquired vWD induced by VAD, with the need for transfusions.

**Methods:** In this retrospective study, 17 patients who had a VAD implant in our hospital in a six-month period were included for analysis. The investigation of underlying vWD was estimated by ristocetin-induced platelet aggregation (RIPA) using classical light transmission aggregometer.

**Results:** Six patients (35.3%) had left-VAD (L-VAD) implantation while the others had biventricular VAD implantation (BiVAD). The mean age was 42.41 years (SD±15.33) and 9 patients (52.9%) were male. Female patients had VAD implantation at younger age than male (p<0.001). The mean follow-up after VAD implantation was 15 months (SD±11.88). At the time of analysis, 13 patients (76.5%) were alive, 2 patients (11.8%) had died while 2 patients (11.8%) had been heart-transplanted. Eight patients (47.1%) had blood type O, 8 patients (47.1%) had blood type A and a patient (5.9%) had AB. Mean RIPA before VAD implantation was 59.3% (SD±14.76) while after VAD implantation was 47.29% (SD±15.47), whereas the decrease was not statistically related. No statistical correlation was found between RIPA among different blood types. Among patients with blood type O, the need for blood transfusions was associated with the duration of having the VAD implantation in months (p<0.001) while the need for fresh frozen plasma (FFP) transfusions was associated with RIPA before VAD implantation (p=0.016). In non-blood O type patients no statistical correlation was found with the need for transfusions with RIPA percentage or median follow-up of patients.

**Summary/Conclusions:** It has been shown by several studies that patients with VAD show a decrease in vWF increasing the bleeding risk. Thus the best antiplatelet treatment and/or anticoagulation that those patient needs, remains challenging. In our study, there was a decrease in mean RIPA percentage after VAD implantation and patients with blood type O had lower RIPA before implantation. However, none of these measurements was statistically significant. The blood type O patients showed an increased need for transfusions in correlation with the duration of VAD implants and an increased need for FFP in correlation with RIPA baseline. Our study has limitations due to the small population and the fact that vWF was not estimated within the different blood groups at baseline and after VAD implantation.
Bone marrow failure syndromes incl. PNH - Clinical

PB1751
ACQUIRED PURE RED CELL APLASIA ASSOCIATED WITH LYMPHOPROLIFERATIVE DISEASES IN ERYTHROPOIETIN-REFRACTORY ANEMIA PATIENTS ON DIALYSIS

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Background: Erythropoietin-refractory anemia is a serious problem and complicated causes should be ruled out in patients on dialysis. Acquired pure red cell aplasia (PRCA) may be hidden behind anemia of chronic kidney disease. Recently it was reported that PRCA patients with large granular lymphocytes frequently had STAT3 mutations (Oie ZY et al. Hematol & Oncol 2013, Ishida F et al. Cancer sci 2014). Molecular or flow-cytometric analysis is useful for detecting a small amount of abnormal lymphocytes.

Aims: We conducted this study to determine the clinical characteristics and STAT3 mutations of patients with acquired PRCA on dialysis with lymphoproliferative diseases.

Methods: In our hospital, 4 patients were diagnosed as having acquired PRCA on dialysis with lymphoproliferative diseases after 2005. Patients were retrospectively studied for presenting feature, laboratory data, and clinical course. Surface markers of lymphocytes were examined by flow cytometric analysis, and T-cell receptor (TCR) rearrangements were examined by Southern blot analysis. Mononuclear cells were separated after obtaining written informed consent. STAT3 (Y640F and D661Y) mutations were examined by allele-specific PCR. Current study was conducted within the guidelines and with the approval of the institutional ethics committee.

Results: In spite of adequate administration of erythropoietin colony-stimulating factor, all 4 patients required blood transfusion due to erythropoietin-refractory anemia. Median leucocyte 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 marker data of 4 patients was as the start of dialysis: 2 patients with low percentage 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.

Summary/Conclusions: A proportion of erythropoietin-refractory anemia patients on dialysis have 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 ANALPSIA PATIENTS

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Background: There is little data about the influence of infection of HBV on the therapy of aplastic anemia.

Aims: This article is aimed at assessment the HBV reactivation risk in HBSAg-positive or HBsAg-negative, antihepatitis B core antigen antibody (anti-HBc) - positive patients with AA receiving CsA and/or ATG.

Methods: We analysis the clinical data of 60 AA patients with HBV infection out of 201 cases of AA from our center at AA diagnosis during the recent 3 years, and laboratory test data such as levels of liver enzyme, HBV DNA in serum, HBsAg anti-HBs and anti-HBc were monitored. Entecavir (ETV) or lamivudine (LAM) was started when HBV reactivation (defined as detectable HBV DNA) was encountered or as a antiviral prophylaxis regimen for some patients.

Results: Among 60(29.8%) AA patients, 12 were chronically infected (HBsAg positive) and 48 were previously exposed (HBsAg negative/anti-HBc positive). 5 patients (8.33%) who were HBsAg positive and not given any riphysicant antiviral therapy suffered HBV reactivation. 7 patients who were HBsAg positive but given were found no HBV reactivation. All the 48 patients with negative HBsAg and positive anti-HBc were found no HBV reactivation during the follow-up.

Summary/Conclusions: Antiviral prophylaxis should be recommended for HBsAg-positive patients who will receive IST with AA as they had high rate (41.6%) of HBV reactivation. HBV infection were found no influence to the clinic course in AA and antiviral therapy had no influence to the effect of IST.

PB1775
MULTICENTER RESULTS OF SCHWACHMAN-DIAMOND SYNDROME PATIENTS

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Background: Schwachman-Diamond syndrome (SDS) is an autosomal recessively inherited disease characterized with neutropenia, exocrine pancreas insufficiency, failure to thrive and skeletal abnormalities. In approximately 90% of the patients, the molecular defect is related to SBD5 gene mutations. The classical triad is present in one-forth of the patients and a high degree of suspicion is required in order to make the diagnosis. In this study, molecular work-up to patients with suspected SDS were made and the clinical and laboratory findings that predict the SDS diagnosis were investigated.

Aims: The aim of the study was to find out the predictive clinical and laboratory characteristics of SDS patients.

Methods: The patients were sent to Hacettepe Inherited Bone Marrow Failure Center for molecular work-up between June 2015 and August 2016. They 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 skeletal dysmorphism and 3 (75%) of the patient had failure to thrive. Three patients (75%) had anemia associated to neutropenia, and 1 patient (25%) had pancytopenia at presentation. On the other hand, all of the patients who were referred with a suspicion of SDS but was found to have no mutation, 43% had neutropenia, 25% had bicytopenia, 10% had pancytopenia. The patients in the latter group had failure to thrive in 25% of the patients and chronic or persistent diarrhea was present in 25% of this group. There was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion. Summary/Conclusions: Although, there was no statistically significant difference in the clinical and laboratory parameters of the genetically verified SDS patients and patients without SDS but was referred with SDS suspicion, this might be attributed to the small sample sizes. Compatible with the previous literature data, SDS is a cryptic disorder and the classical triad is not commonly fulfilled in most of the patients. On the other hand, failure to thrive/growth retardation was three times more common in patients with SDS. Thus, in patients neutropenia, accompanying failure to thrive/growth retardation might be an indicative to make molecular work-up for SDS. Additionally, not only neutropenia, but bicytopenia or pancytopenia might be the hematological presentational findings of SDS.

PB1755 PAROXYSMAL NOCTURNAL HEMOGLOBINURIA AND APLASTIC ANAEMIA – DATA FROM THE SPANISH PNH REGISTRY
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Background: Aplastic anaemia (AA) and Paroxysmal Nocturnal Hemoglobinuria (PNH) are included, together with other pathologies, within the bone marrow failure syndromes. In the present study, these clinical entities were 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 patients were asked. Soon after, a descriptive analysis of the data was performed.

Results: 34 patients were recruited and analyzed (12 women and 22 men). Their age interval ranged from 2 to 87 years, and all of the patients suffered from either PNH with pancytopenia and/or AA with a developing PNH clone. The average age at the time of initial diagnosis was 28.5 years old (4m-72y).

In all, 15 patients (44%) had PNH clone at diagnosis (65-99%). Treatment response with eculizumab, generally obtaining a very good response. In at least half of the cases, making it necessary in these patients treatment with eculizumab, generally obtaining a very good response.

Summary/Conclusions: Clonal evolution in AA is frequently associated with the development of a PNH clone at the time of diagnosis, throughout the pathology natural course or even after disease’s resolution. The development of such clone has been related to better prognosis in AA right after the immunosuppressive therapy (IST). Our experience demonstrated the presence of hemolysis in at least half of the cases, making it necessary in these patients treatment with eculizumab, generally obtaining a very good response.

PB1756 AUTOIMMUNE CYTOPENIAS IN PRIMARY IMMUNODEFICIENCY DISEASES: SINGLE CENTER EXPERIENCE
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Aims: Primary immunodeficiency diseases (PID) 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 8 patients (5% of the patients). In the patients treated with eculizumab, a very good response was observed.

Results: In the patients treated with PID and autoimmune cytopenias. 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 importance of autoimmune cytopenias as a possible complication of PID.

PB1757 HEAVY METAL LEVELS IN PATIENTS WITH FANCONI APLASTIC ANEMIA
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Background: Fanconi aplastic anemia (FAA) is a rare, autosomal recessively inherited bone marrow failure syndrome. Patients with 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 control group. Plasma copper (Cu), iron (Fe), zinc (Zn), chromium (Cr), cobalt (Co), selenium (Se) levels were measured in patients with FAA.

Results: Total of 17 patients with FAA were included in the study. Median age was 9 years (1-30), female to male ratio was 8/9. One patient had undergone stem cell transplantation, four patients were trangression 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, five patients died because of immunodeficiency.

Table 1. Heavy metal levels in patients and control group.

Summary/Conclusions: There is a paradoxal situation between PID and autoimmunity. The reduction of central and peripheral tolerance is held responsible for autoimmunity in PID.

Table 1. Heavy metal levels in patients and control group.

Data from the Spanish PNH Registry.

FAA; Fanconi aplastic anemia.

PB1758 AUTOIMMUNE CYTOPENIAS IN PRIMARY IMMUNODEFICIENCY DISEASES: SINGLE CENTER EXPERIENCE
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Aims: Primary immunodeficiency diseases (PID) 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 8 patients (5% of the patients). In the patients treated with eculizumab, a very good response was observed.

Results: In the patients treated with PID and autoimmune cytopenias. 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 importance of autoimmune cytopenias as a possible complication of PID.
Summary/Conclusions: In our study we found chromium and cobalt levels higher in patients with FAA than control group. In vitro studies have revealed that FAA cells are more sensitive to chromium toxicity. With larger number of patients chromium level and clinical association should be investigated in further studies.

Results: 

- Median follow up was 7.2 years. Both age equal or older than 65 years and PNH with high disease activity were associated to poorer overall survival (HR: 0.0134 and 0.045 respectively). Regarding PNH with high disease activity, 18 patients were identified, 4 of them in geriatric age. In 2 of them (50%), CIRS-G >10 were associated to poorer overall survival (HR: 0.0134 and 0.045 respectively).

PB1758

CLINICAL IMPACT OF AGE AND COMORBIDITY IN PNH PATIENTS

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Background: PNH is an ultra-rare disorder affecting mainly young adults, but can be diagnosed in geriatric population. Comorbidity is more prevalent in geriatric population and can either hamper diagnostic evaluation or increase the complexity of PNH patient care.

Aims: To identify geriatric-age PNH in Spanish PNH registry. To study the clinical characteristics at diagnosis and evolution of geriatric-age PNH and compare them to non-geriatric PNH population. To analyse the impact of both age and 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 v5 (La Jolla, CA).

Results: 

- 44 patients from 11 centres in Spain have been included up to date. 8 patients (17.8%) were diagnosed in geriatric age (equal or older than 65 years). Age range for the complete cohort: 17-83 years and 9 patients presented with high comorbidity, arbitrary defined as CIRS-G score >10. (Range for the 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 neoplasm), 3 of them (75%) in geriatric age. Median follow up was 7.2 years. Both age equal or older than 65 years and CIRS-G >10 were associated to poorer overall survival (HR: 0.0134 and 0.045 & p = 0.0015 and 0.0103 respectively). Regarding PNH with high disease activity, 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-NAIVE 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).

Aims: To identify clinical and laboratory features that predict disease progression, time to treatment and survival in treatment-naïve patients with +12 CLL.

Methods: A study included 487 treatment-naive 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 unmutated IGHV remained significantly associated with shorter PFS, TFS, OS and CLL-specific survival, higher Rai stage with shorter PFS and elevated β-2-microglobulin with shorter OS. Considering interestingly the association of a simple and new laboratory parameter such as LDH to the outcomes, confirmed on multivariate analyses for FISH (hazard ratio [HR] 1.55, 95% confidence interval [CI] 1.2-2.0; p=0.002), TFS (HR 1.22, 95% CI 1.2-2.2; p=0.002), OS (HR 1.69, 95% CI 1.2-2.7; p=0.034) and CLL-specific survival (HR 3.86, 95% CI 2.0-7.5; p=0.001), we divided our +12 CLL cohort according to LDH levels available at diagnosis: 103 patients showed LDH levels above the normal limit and 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.

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 determined by dividing the variable into deciles, and selecting the most efficient cut-point, and based on previous publications.

Results: FISH detected aberrations in 85% of the cases (442/500). The most frequent abnormality was 13q-, observed in 302 patients (47%), but as a sole alteration in 212 cases, followed by +12 (106 patients, 16%), 11q- (83 patients, 13%), and 17p- (33 patients, 5%). As expected, the group of patients with 13q- as a sole abnormality was the one with the better OS (195 months) followed by the group of patients with normal FISH (160 months), +12 (124 months), 11q- (56 months) and 17p- (46 months), consistent with the Döhner hierarchical classification (Döhner H et al. NEJM 2000). Similar results were observed in TTFT: 13q- as sole abnormality (106 months), normal FISH (112 months), +12 (29 months), 11q- (10 months), 17p- (10 months). The best predictive cut-off point that divided patients according to its prognosis was different for each alteration. We confirmed that a high percentage of cells carrying the deletion is associated with a significantly worse TTFT in cases with 17p, 13q, and 11q deletions, and a significantly shorter OS in cases with 17p deletion. We observed a similar trend for OS in cases with 13q and 11q deletions, probably not significant because of the low number of patients included, compared to previous studies. We observed the same trend in patients with +12. The Table 1 summarizes these findings. Probably with a higher number of cases and a longer follow up, it could have also been possible to reach statistically significant differences in the subgroups in which it was not objected.

<table>
<thead>
<tr>
<th>FISH abnormality</th>
<th>Number of cases</th>
<th>Overall survival (months)</th>
<th>P</th>
<th>Time to first treatment (months)</th>
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<tr>
<td>13q deletion</td>
<td>302</td>
<td>195</td>
<td>0.001</td>
<td>212</td>
</tr>
<tr>
<td>11q deletion</td>
<td>83</td>
<td>160</td>
<td>0.001</td>
<td>30</td>
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<tr>
<td>17p deletion</td>
<td>33</td>
<td>46</td>
<td>0.001</td>
<td>56</td>
</tr>
<tr>
<td>+12</td>
<td>106</td>
<td>124</td>
<td>0.001</td>
<td>29</td>
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<tr>
<td>Normal FISH</td>
<td>249</td>
<td>112</td>
<td>0.001</td>
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</tbody>
</table>

Summary/Conclusions: Not only the type of cytogenetic abnormality but also the percentage of abnormal nuclei detected by FISH are important factors in the prognosis of CLL patients.
METHYLATION STATUS OF RAD21 GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic Lymphocytic Leukemia (CLL) pathogenic mechanisms have not been fully elucidated yet. However, genetic and epigenetic alterations seem to be involved in the pathogenesis and extensive clinical heterogeneity of the disease. DNA methylation in CpG sites of a gene promoter, which affect the chromatin structure as well as gene transcriptional activity, is a crucial epigenetic modification in CLL. RAD21 gene is involved in DNA repair and its encoded product acts as basic subunit of the Cohesin protein complex that regulates the cohesion and proper separation of sister chromatids during mitosis or meiosis.

Aims: We investigated the methylation status of RAD21 gene promoter and its possible implication in CLL pathogenesis and the formation of CLL cytogenetic aberrations.

Methods: The study included 105 CLL patients and 17 healthy donors (controls). Total genomic DNA extraction was performed from bone marrow or peripheral blood samples of all patients and controls. Methylation analysis of RAD21 gene promoter was carried out using the new technology of MethylScreen™ in the CFX96Biorad Real-Time PCR system. For this purpose, we used EpTect Methyl II PCR Assay which enables us to calculate the methylated and unmethylated fraction after simultaneous digestions with specific restriction endonuclease. Methylation analysis was performed on unstimulated and stimulated with CpG-oligonucleotide DSP-30 bone marrow cells of CLL patients. FISH analysis was carried out using the commercial CLL set probes for detection of the most common abnormalities of the disease including deletions of 17p13 (TP53), 11q22.3 (ATM) and 13q14.33/13q34 (D13S319/13q34) regions and trisomy 12 (CEP 12).

Results: Among the 105 CLL patients, 21 patients exhibited a normal karyotype also confirmed by FISH and 84 patients showed chromosome abnormalities detected by karyotypic or/FISH analysis. Methylation study was successful in all healthy donors and in 101 out of 105 CLL patients. All healthy donors had normal methylation of RAD21 gene promoter. On the contrary, 25.74% (26/101) of CLL patients carriers >10% cells with methylated CpG islands in RAD21 promoter, which was significantly increased compared to controls (p=0.039, χ²=4.25, df=1). RAD21 methylated cell fraction varied among patients. More specifically, 9.9% of patients (10/101) showed 11-50% methylation rate, 10.89% (11/101) 51-89% 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 karyotype and 25% of patients (20/80) with abnormal karyotype. In detail, methylation in RAD21 promoter was present in 33.33% of patients with trisomy 12 (n=14/42), in 33.33% (4/12) with trisomy 8, in 31.25% (5/16) with -17del(17p), in 27.78% (5/18) with trisomy 15, in 25.81% (8/31) with del(13q), in 20% (2/10) with del(6q) and in 12.5% (2/16) with del(11q). Based on karyotypic complexity, RAD21 promoter was methylated in 18.18% (4/22) of patients with a single chromosome aberration, 26.09% (6/23) with two chromosome aberrations and 25.71% (9/35) of patients with complex karyotype (>3 aberrations).

Summary/Conclusions: Methylation of RAD21 gene promoter, which leads to transcriptional inactivation and consequently inhibition of RAD21 expression, seems to be implicated in CLL pathogenesis and the formation of specific chromosome aberrations. Clarification of the epigenetic landscape of CLL may help in the design of new targeted therapeutic agents.

ROLE OF KEAP1-NRF2 PATHWAY GENETIC VARIABILITY IN THE SUSCEPTIBILITY AND PROGNOSIS OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA
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Background: Chronic lymphocytic leukemia (CLL) is the most prevalent leukemia in the western adult population. Although advanced age, white ancestry, and family history of hematologic malignancies are risk factors, the etiology of CLL still unknown. One of the mechanisms associated with the development of this pathology is related to the oxidative stress (OS) resulting from an imbalance between the production of reactive oxygen species (ROS) and their disposal by the antioxidant defenses. The number factor erythroid 2-like gene 1 (NFE2L2) and its suppressor, the Kelch-like ECH-associated protein 1 (KEAP1) gene, plays a central role in ROS balance. Changes in these genes, whether due to somatic mutations or genetic variants (SNPs), have been associated with some hematological diseases. However, the role of NFE2L2 and KEAP1 genes polymorphisms in susceptibility and prognosis of CLL has not been studied.

Aims: To assess the role of two SNPs in the NFE2L2 and KEAP1 genes on CLL susceptibility, their influence on prognosis/survival, and their correlation with clinical and laboratory characteristics of patients.

Methods: Genetic variants rs13001694 (NFE2L2) and rs11085735 (KEAP1) were genotyped by tetra-primers-AMPS-PCR in 176 patients with CLL and 261 controls. The role of these genes polymorphisms in CLL susceptibility and their association with clinical and laboratory characteristics as well as with therapy response was assessed by logistic regression analysis and/or by Fisher’s exact test. The influence on prognosis and survival was performed through Kaplan-Meier analysis and/or by the log-rank test by estimating the progression free survival (PFS) and the overall survival (OS).

Results: The results showed that individuals with the GG genotype (NFE2L2) are at higher risk of developing CLL (Odds ratio (OR) = 2.032; 95% confidence interval (CI): 1.234-3.51; P<0.004). In addition, the genotypic profile (GP) GG / CC (NFE2L2/KEAP1) is a risk factor (OR: 2.186; 95% CI: 1.273-3.744; p=0.003) for the development of CLL while the AA / CC profile constitutes a protective factor (OR: 0.634, 95% CI: 0.407-0.984, p=0.037). In contrast, patients with genotype AG (NFE2L2) and/or CC (KEAP1) had a higher rate of complete response to rituximab therapy regimens (NFE2L2 AG: OR 1.6; 95% CI 1.063-2.331, p=0.037; KEAP1 CC OR 1.2, 95% CI 1.041-3.477, p=0.045, NFE2L2 / KEAP1 AG / CC: OR: 1.9, 95% CI, 1.843-4.485, p=0.017) and with fludarabine (NFE2L2 / KEAP1 AG / CC: OR 1.5, 95% CI, 1.119-3.887, p=0.026). Finally, the overall survival of CLL patients appears to be influenced by the genotypic profile of NFE2L2 / KEAP1 (GP AG / AC patients have a lower mean survival of 13 months vs. 18.1 months of patients with other GPs, p=0.037), while progression-free survival seems to be influenced by the KEAP1 genotype [patients with CC genotype have a longer mean survival (198.0±13.6 months) than patients with AA and AC genotypes (85.3±13.4 months; p=0.023)].

Summary/Conclusions: This study suggest that genetic polymorphisms in NFE2L2 and KEAP1 genes might be risk factors for CLL development and may constitute novel genetic markers for therapy response (namely regimes with rituximab and fludarabine) as well as prognostic markers, by influencing overall survival and progression free survival in CLL patients.

The authors declare no conflicts of interest.
Background: Chemokines and their receptors are involved in the regulation of cell recruitment, survival, proliferation, and trafficking, all these processes crucial in the pathogenesis of chronic lymphocytic leukemia (CLL). Comprehensive profiling of chemokine receptors in CLL and its subgroups according to prognostic relevance is missing.

Aims: To characterize the chemokine expression pattern in CLL patients and subgroups according to clinical course and cytogenetic aberrations.

Methods: We studied the gene expression pattern of 16 canonical and 4 atypical chemokine receptors in peripheral blood mononuclear cells (PBMC) of CLL patients (n=88) and healthy subjects (n=34) by using SmartChip quantitative RT-PCR (WalterGen Bio-systems). The expression of CX3CR1, CX4R5, CXCR5, CXCR7, and CCR7 was confirmed by flow cytometry.

Results: Among deregulated receptors, 5 receptors (CCR7, CCR10, CX3CR1, CXCR4, CXCR5) were up-regulated and 9 receptors (CCR2-CCR6, CCR8, CXCL12, CXCL14) were down-regulated in CLL. The expression of CCR2-CCR6 and CXCL12-CXCL14 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 CCR7 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.
PB1768

ACTIVITY OF THE CD19 ANTIBODY MOR208 IN COMBINATION WITH IBRUTINIB, IDELALISIB OR VENETOCLAX IN VITRO

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Background: CD19 is broadly expressed across B-cell malignancies, including chronic lymphocytic leukemia (CLL). MOR208 is an Fc-enhanced CD19 antibody mediating potent antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (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), ibritinib and idelisib, and the BCL-2 inhibitor, venetoclax.

Methods: The CLL cell line MEC-1 was treated with 0.3–10 µM ibritinib, idealisib 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 antiprofisive or cytotoxic effects of the TKIs or venetoclax. Dose-dependent ADCC activity of MOR208 was analyzed by flow cytometry. Cytotoxic effects were studied in at least three independent experiments.

Results: Ibritinib and idelisib induced only moderate direct cytotoxicity on ME1 target cells but had strong antiproliferative effects. In contrast, venetoclax induced strong cytotoxicity on ME1 target cells within 24 hours. Both effects led to reduced tumor target cell numbers in the subsequent ADCC assays. CD19 expression was largely unaffected by all three drugs. The addition of MOR208 to ibritinib, idealisib 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 ibritinib, 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 accumulation 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) are major inhibitory receptors and factors of B-cell Chronic Lymphocytic Leukemia (CLL) B cells.

Aims: We conducted a study to determine the percentages and absolute numbers of exhausted lymphocytes B and T in peripheral blood and bone marrow of CLL patients. Moreover, we analyzed relationship between the number of PD-1-positive, PD-1+CD20+ cells and expression of HSP70 and HSF1 protein in CLL patients following Epstein-Barr Virus (EBV) infection and after ibrutinib therapy.

Methods: The study included 60 untreated patients with CLL and 20 healthy subjects. The immunophenotype of peripheral blood mononuclear cells (in both groups) and bone marrow cells (solely in the CLL group) was determined by means of flow cytometry.

Results: Patients with CLL showed higher frequencies and absolute number of exhausted B lymphocytes CD19+PD-1+ (p<0.0001), CD19+PD-L1+ (p=0.0001), CD19+CD200+ (p<0.0001) and CD19+CD200R+ (p<0.0001), as well as higher frequencies and absolute number of exhausted T helper lymphocytes CD4+PD-1+ (p=0.0021), CD4+PD-L1+ (p=0.0032), CD4+CD200+ (p=0.0027), CD4+CD200R+ (p=0.0056), and exhausted T cytotoxic lymphocytes CD8+PD-1+ (p=0.0036), CD8+PD-L1+ (p=0.0029), CD8+CD200+ (p=0.0038), CD8+CD200R+ (p=0.0073) than the controls in the peripheral blood. Similar observations were done in the bone marrow samples (p<0.0001; p<0.0001; p<0.0001; p=0.0134; p=0.0183; p=0.0263; p=0.0169; p=0.0261; p=0.0362; p=0.0293, and p=0.0379, respectively). Enhanced exhaustion of peripheral blood and bone marrow lymphocytes was associated with higher Rai stage, increased concentration of lactate dehydrogenase and beta-2 microglobulin, and more rapid progression of the disease. The number of lymphocytes B CD19+ZAP-70+ correlated positively with the number of CD19+PD-1+ B cells, CD4+PD-1+ T cells, and CD8+CD200+ T cells.

Summary/Conclusions: The study confirmed the association between unfavorable prognosis and high expression of exhaustion markers in CLL patients. Determination of PD-1+, PD-L1+, CD200+, and CD200R+ lymphocytes and their ratios constitutes valuable diagnostic tool, completing cytometric evaluation of CLL.

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 neoplastic disorder characterized by the accumulation of clonal B cells in peripheral blood, bone marrow and lymphoid tissues. CLL is a clinically and biologically heterogeneous disease. As a consequence, novel biological and cytogenetic features have become increasingly important in predicting prognosis at the time of diagnosis and the research for molecules involved in apoptosis resistance and increased survival of neoplastic B cells is still ongoing.

Aims: We recently found that the Heat Shock Protein of 70kDa (HSP70) is overexpressed in Chronic Lymphocytic Leukemia (CLL) B cells. Considering the role of HSP70 in cancer, we were aimed at characterizing this protein and its master regulator, the Heat Shock Factor 1 (HSF1), within the pathogenetic mechanisms leading to CLL.

Methods: HSP70 and HSF1 expression levels were evaluated by Western blotting (WB) analysis in leukemic and normal B cells. HSP70 and HSF1 protein levels were correlated 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 B 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 progresses have been made in bringing HSP70 inhibitors to the clinic, because of their potential off-target effects. For this reason we tried an alternative approach by targeting the HSP70 major regulator, HSF1. We observed that both inhibitors, Zafirlukast and Fisetin, lead to an in vitro dose dependent cell apoptosis. These data demonstrate HSP70 and HSF1 involvement in the pathogenesis of CLL and identify HSP70/HSF1 axis as a target for new therapeutic strategies.

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 and biological and molecular features, as well as standard laboratory parameters. The expression of SLC28A3 gene was analyzed in peripheral blood mononuclear cells by RT-PCR methodology, using TaqMan chemistry and ABL as endogenous control gene. Quantification of target gene expression was made by comparative ddCt method using HLA-BL cell line as the calibrator. All analyses were done prior to any treatment.

Results: Median age at diagnosis was 57 years (range 38-75). All patients were treated with fludarabine-based chemotherapy, 43 (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 73 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 mutation status. Patients who experienced PD to FC treatment overexpressed gene for hCNT3 compared to patients who achieved CR (p=0.013) and PR (p=0.05). We detected a significantly higher level of SLC28A3 expression in patients who experienced PD to FC treatment in comparison to patients who achieved CR (p=0.013) and PR (p=0.05).

Summary/Conclusions: Overexpression of SLC28A3 gene is a predictor of resistance to treatment with FC chemotherapy. Further studies are warranted to confirm these findings.

PB1772

THE SPECTRUM OF TP53, SF3B1, AND NOTCH1 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS EXPOSED TO IONIZING RADIATION DUE TO THE CHORNObYL NPP ACCIDENT

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Background: Generally, chronic lymphocytic leukaemia (CLL) is considered to be a non-radiogenic form of leukaemia. 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 tumour and Richter transformation, mainly unmutated status of heavy chain variable region (IGHV) genes with increased usage of IGHV1-69 and IGHV3-21 (Abramenko et al., 2008). Analysis of genetic features of leukemic cells in IR-exposed CLL patients may provide an additional data on the possible causal relationship with IR.

Aims: The aim of the study was to analyze TP53, NOTCH1 and SF3B1 mutations in CLL patients, sufferers of Chornobyl NPP accident to clarify the possible pathogenetic relationship between IR and CLL development.

Methods: TP53, NOTCH1, and SF3B1 mutations were analyzed in 106 CLL patients who have been exposed to ionizing radiation (IR) due to Chornobyl NPP accident (83 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 hotspot regions of these genes were the vast majority of CLL-specific lesions were reported: in c.711G>A (n=4), c.711A>G (n=1), c.1609delC (n=1), c.1146G>A (n=1) and c.1669delT (n=1), respectively.

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 identified 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). Other specific 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 clonal event evolving into tumor heterogeneity. We focus on Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma and Folicular lymphoma (FL) that are currently considered incurable. Though current treatment regimen includes standard chemotherapies, CLL and MM cancer eventually relapse. Current challenges in using therapies against CLL and MM includes design of optimal treatment for individual patients based on characterization the tumor and its intratumor heterogeneity as observed by whole genome sequencing. Efficient therapies require a personalized approach that combines targeting lymphoma cells and the tumor microenvironment by restoring the patient’s own anti-tumor immunity. One solution to this challenge is the so-called “n-of-one” studies where protocols are organized with diagnosed 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 CD40L, 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 T-helper Cells prestimulation in the presence of IL2. To support high throughput drug sensitivity screening. We use cell-based assays such as CellTiter-Glo®, CellTiter-Fluor® 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 (unsimulated and soluble CD40 ligand stimulated) has been performed 48, 72, 96 and 120 hrs (days) using cell proliferation, CellTox-Green assay for M2 cells has been performed. Benzalkonium chloride (BzCl) is used as Positive control. Endpoint measurement using CellTiter-Glo assay for CLL and MM cells was performed with cell density of 5000. Dose Response curve for 50 drugs has been generated for CLL patients (n=4) and MM (n=4) (Figure 1).

Summary/Conclusions: We perform drug sensitivity screening to select potential drug candidates and pathway inhibitors through an approach where we directly assess patient samples. Selected drug candidates will first be validated by biopsays and the flow cytometry to assess effects on intracellular mitogenic pathways (phosphoflow-based approach). We propose to use the drug sensitivity screening platform to identify and validate drug candidates for xenografting and “n-of-one” clinical trial studies.

Figure 1.
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 shaping of the CLL IGHV gene usage is influenced by different genetic and environmental factors. In addition, a strong bias in the use of individual genes and subgroups between normal and malignant B-cells and presence of highly homologous “stereotyped” heavy complementary-determining region 3 (VH-CD3) is shown, which suggests the role of a specific antigen in the pathogenesis of the disease.

Aims: In this study, we analyzed the mutation status and pattern of IGHV,IGHD and IGHJ gene usage in Macedonian CLL patients. Methods: Ninety-seven consecutive CLL patients that presented at the University Clinic of Hematology –Skopje in the period between 2011-2013, were included in the study. IGHV mutation status and gene repertoire were analyzed using the reverse transcriptase– polymerase chain reaction (RT-PCR) and sequencing methodology. The mutational status of the 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).

Most frequently expressed IGHV genes were: IGHV3 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 IGHD gene was IGHD4 (49.4\%), followed by IGHD8 (23.9\%), IGHD1 (13.4\%), IGHD3 (11.4\%) and IGHD2 (13\%), IGHD1 (2.06\%). In 10\% of the cases, the VHCD3 amino acid sequences belong to previously defined stereotyped clusters. Only one of the rearrangements with stereotyped VH-CD3 belonged to the M-CLL subset.

Summary/Conclusions: Our study showed a strong correlation between IGHV gene mutational status and clinical course of CLL. Results on IGHV-IGHD-IGHJ genes usage in our study are comparable to the previously reported from Mediterranean countries. The high frequency of V1-69gene and low frequency of IGHV3-21 in our CLL patients that originate from a small geographic region further promotes the geographic bias in the use of 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.

PB1774

FCGR2A AND FCGR3A VARIANTS ARE NOT ASSOCIATED WITH RITUXIMAB IN PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Methods: We analyzed these two polymorphisms in 90 patients with CLL treated with R-FC regimen. Median age of our patients was 62.7(36-78) and 63\% were male. Number of patients with stage III/IV disease was 65(72\%) and median WBC count at the start of treatment was 68.5(34-173x109/L). Percentages of previously treated patients was 51/90 (56.6\%). Average numbers of Fc 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.

Results: 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=0.8001) or FCGR3A (p=0.1019) in CLL patients with complete, partial or no response to R-FC therapy (Table 1).

Table 1. Genotype distributions for FCGR2A & FCGR3A in patients with CLL.

<table>
<thead>
<tr>
<th>FCGR2A/FCGR3A</th>
<th>Complete Response (n=24/60.7%)</th>
<th>Partial Response (n=50/62.2%)</th>
<th>No Response (n=16/17.7%)</th>
<th>p-value</th>
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<tr>
<td>FCGR2A H131R</td>
<td>R(20.6%)</td>
<td>10(20.0%)</td>
<td>4(13.5%)</td>
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<td>(131H/R)</td>
<td>(131H/H)</td>
<td>(131R/R)</td>
<td>(131R/H)</td>
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<tr>
<td>(131H/H)</td>
<td>6(27.5%)</td>
<td>5(26.3%)</td>
<td>9(56.3%)</td>
<td></td>
</tr>
<tr>
<td>FCGR3A 158V/V</td>
<td>R(20.5%)</td>
<td>10(26.3%)</td>
<td>4(16.7%)</td>
<td>0.010</td>
</tr>
<tr>
<td>(158 F/F)</td>
<td>(158 F/V)</td>
<td>(158 V/V)</td>
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<td>11(33.3%)</td>
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Summary/Conclusions: Our results are similar with previously reported results in other studies in CLL patients, but in contrast with the results for follicular lymphoma (FL), which showed that high-affinity FCGR2A-158V/V variant was associated with the highest response rates in FL patients treated with rituximab. These findings could be explained with the different mechanism of action of rituximab in CLL compared to lymphoma patients or could be due to the variations in selected patient’s population.

PB1775

MUTATIONAL STATUS, IMMUNOGLOBULIN HEAVY VARIABLE GENES PATTERN AND STEREOTYPED RECEPTORS REPERTOIRE OF MACEDONIAN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Chronic lymphocytic leukemia and related disorders

**Clinical**

**PB1776**

**LAMBDA LIGHT CHAIN RESTRICTION – USEFUL FOR HAIRY CELL LEUKEMIA PROGNOSTICATION?**

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**Background:** Hairy cell leukemia (HCL) patients have near-normal life expectancies since the introduction of purine nucleoside analogues. However, HCL remains a chronic, often relapsing disease in which maximizing treatment-free survival (TFS) is the main goal.

**Aims:** Prognostication is not standardized in HCL, emphasizing the relevance of the characterization of HCL populations.

**Methods:** We retrospectively analysed 40 patients (90% men), diagnosed between 1997 and 2016, with a median follow-up of 6 years.

**Results:** At presentation, the median age was 58 years and 69% of patients were symptomatic - fatigue (53%), B symptoms (50%), bleeding (14%), abdominal discomfort (6%) and severe infection (22%). The commonest cytopenia was thrombocytopenia (70%), with median platelet count being 66x10^9/L. Monocyte counts below 0.1x10^9/L were observed in 61% of patients. Splenomegaly was observed in 83% of the patients and 21% had abdominal lymphadenopathies. The majority of the patients (88%) was treated with cladribine in first line, achieving an overall response (OR) rate of 100% and a complete response (CR) rate of 38%, of which 67% were classified as minimal residual disease (MRD)-negative CR. Retreatment was required in 33% of the patients, of which the majority received cladribine. The median time-to-next-treatment (TNT) from first to second line treatment was 3 years. The OR rate for second-line treatment was 91%, 50% achieving CR, of which 33% were classified as MRD-negative CR. Only 5% of the patients required further treatment lines. Even the presence of scarce hairy cells in the bone marrow precluded classification of response as CR. This might have contributed to the low CR levels observed in our patients. As post-treatment bone marrow biopsies were available in only 24 patients, response analysis was restricted to these patients. All of these 24 patients had bone marrow fibrosis at diagnosis, which reverted when and in whom first CR was obtained. Median overall survival (OS) was not reached and, after 10 years, the OS was 90%. Four deaths occurred, all unrelated to HCL. Considering prognostication, a trend to a longer TFS, albeit not statistically significant, was observed in patients achieving CR (namely MRD negative) and without thrombocytopenia at presentation. Excitingly, the 61% of patients with kappa (κ) light-chain restriction (LCR) displayed a significantly higher TFS than the 39% with lambda (λ) LCR (p=0.04, Wilcoxon-Gehan test). To the best of our knowledge, there are no published reports on prognostic value of LCR in HCL (Figure 1).

**Summary/Conclusions:** If multicentre studies corroborate our findings, LCR may be of use in the prognostication/risk stratification of HCL. Similarly with multiple myeloma and other hematological malignancies, lambda (λ) LCR appears to correlate with worse prognosis, leading to a shorter TFS.

**Fig. 1.**

**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**

**MONOCYCLONAL B-CELL LYMPHOCYTOSIS IN THAI POPULATION: PREVALENCE AND IMMUNOPHENOTYPIC CHARACTERISTICS**

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**Background:** Monoclonal B-cell lymphocytosis (MBL) is characterized by the presence of <5X10^9 clonal B-cells/L in peripheral blood (PB) in otherwise healthy subjects, in the absence of symptoms and signs of a B-cell lymphoproliferative disorder (LPD). MBL is considered a precursor to chronic lymphocytic leukemia (CLL) and other B-cell malignancies.

**Aims:** To study the immunophenotypic features and prevalence of MBL in healthy Thai individuals.

**Methods:** Peripheral blood (PB) samples from 616 healthy Thai individuals (313 female), 18-80 year-old with normal lymphocyte counts were immunophenotyped using high-sensitive flow cytometry, based on 5-color flow cytometry with the screening for >5 x 10^9 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 CD5, CD23, CD20 and CD79a expression.

**Results:** Of total 616 subjects, MBL was found in 8 cases (1.2%) including 3 and 5 male and female cases respectively. Among 40 years or older, MBL was found in 5 out of 448 cases (1.1%). Compared with non-MBL group, subjects with MBL were significantly older (median age: 57 years vs. 50 years; p=0.01) and had a significant higher number of absolute and B-lymphocyte count (median 3.1 versus 1.6 x 10^9/L; p=0.03 and 0.35 versus 0.16 x 10^9/L; p=0.02, respectively) while the median white blood cell count was not different between 2 groups. Also, there were more subjects in MBL group who had family history of lymphomatoses vs diseases (LPD, 37% vs 0%; p=0.01) and influenza vaccination within 2 years (50% vs 8.7%; p=0.003). Among 8 cases with MBL clone, 6 cases had low-count MBL (<0.5X10^9 clonal B-cells/L) while only 2 cases had high-count MBL (>0.5X10^9 clonal B-cells/L). All 8 cases had persistent positivity of MBL clone after tested was repeated within 3 months after the initial test. In follow up test, only 1 case with initial high-count MBL had decrease number of B cell clone and became low count MBL. There was not significant different in age between subjects in low and high-count MBL group. Six cases had typical CLL phenotype MBL clone (CD5+, CD23+, CD20+/dim and light chain restriction). Whereas 1 case had atypical CLL phenotype MBL (CD5+, CD23+, CD20+/bright and heavy chain restriction). In univariate analysis, age (RR 4.19; 95%CI 1.0-17.7; p=0.049), absolute lymphocyte count (RR 2.76; 95%CI 1.01-4.87; p=0.047), family history of LPD (RR 12.2; 95%CI 5.1-29.3; p<0.001) and

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influenza vaccination (RR 10.47; 95%CI 2.54-43.07; p<0.003) were associated with increased risk of developing MBL. After adjusted for age, only history of influenza vaccination and family history of LPD were an independent risk factor for developing MBL with age adjusted RR of 9.75 (95%CI 2.3-40.5; p=0.002) and 92 (95%CI 56.3-149.5; p<0.001), respectively.

Summary/Conclusions: MBL prevalence in Thai population is much lower than previously reported. It more frequent in elderlies and associated with family history of LPD and influenza vaccination. Although uncommon, the presence of high-count MBL warrants further investigations to define the biological and clinical significance of 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 clinical-biologic features of 9 Binet stage A CLL patients from our Center in Rome who experienced a persistent spontaneous clinical regression of the disease at a median time of 11 years to the last follow-up. In 5/9 cases 5 months at diagnosis. 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, often with VH3-30 and VH3-21. There were 9 patients; 6 males and 3 females, with a median age of 57 years at diagnosis (range 51-82), stage Binet/A1 in 6, A2 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 8/3 patients with VHH-30 (2), VH3-21, VH3-15, VH3-24, VH3-31, VH4-34, VH4-59; CD38 -30% in 6/6; ZAP70 <20% in 4/6; FISH (7 cases): del 13q in 1, del 17p in 1, del 20q in 1. No patient had undergone treatment except for one diagnosed in 2009 who received FCR for disease progression in 2013 (lymphocytes 107 x 10^9/L), obtained a PR and 18 months later developed a Richter’s syndrome - a diffuse large B-cell lymphoma clonally unrelated to CLL - with the concomitant disappearance of the CLL clone from the PB and bone marrow, that has lasted up to January 2017 (lymphocytes 3.5 x 10^9/L). CLL 0.035 x 10^9/L). An additional case diagnosed in 2013 (stage A1, lymphocytes 37.2 x 10^9/L) reached the highest lymphocyte count 19 months later (91.2 x 10^9/L) and subsequently started a spontaneous reduction in lymphocyte count down to 39.6 x 10^9/L in 2015 and to 8.9 x 10^9/L in January 2017 in stage C1. PR was observed. Exclusion criteria were: CLL with bone marrow involvement, last cases, in the other 7, all in stage A0, the highest lymphocyte count was 16.0 x 10^9/L (8.9-76.0), the lowest at the last follow-up was 2.8 x 10^9/L (1.8-4.4), with 0.66 x 10^9/L. CLL cells (0.085-3.0) in the 4 evaluable cases. The median time from diagnosis to clinical regression was 4 years (range 2-17) and have been reported for 2 further cases (range 0.5-7). One of these cases (mutated VH3-21, +12) seems the most dramatic: in 2008 at diagnosis, the lymphocytes were 51.9 x 10^9/L, in 2009 a peak at 76.0 x 10^9/L was recorded; in 2011, when the CLL regression started, the patient underwent several mild viral upper respiratory infections; the CLL complete regression (1.8 x 10^9/L) persisted and was confirmed by blood and bone marrow aspiration. The patient, at the age of 84 years, had a cerebral hemorrhage, a stroke, a pelvis fracture and a Richter’s syndrome - a diffuse large B-cell lymphoma clonally unrelated to the CLL, as could explain, at least partially, the mild clinical phenotype in treated pts. The assessment of platelet function in IBR treated CLL pts could help to predict and monitor the bleeding risk, and to guide pts through invasive procedures. In addition, pts under anticoagulant or antiplatelet treatment might need be carefully monitored by clinical and laboratory evaluation.
Background: Hairy cell leukemia (HCL) accounts for approximately 2% of all leukemias and is associated with pancytopenia, splenomegaly, and recurrent infections. Therapy with the purine analogues cladribine (2CdA) or pentostatin (2’deoxycoformycin), has been most effective and both agents have achieved equivalent results in HCL. In this regard cladribine given as a single course, achieves a high response rate. Several alternative dosing schedules have been reported. Hereafter, we present the results of a single institution experience with cladribine in a consecutive series of patients 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 90.62% respectively. The median age at diagnosis was 53 years, and most (81.77%) were males. In terms of ethnicity: 88.3% of patients were Jews (52.2% Ashkenazi and 36.1% Sephardic Jews) while 11.7% were Arab, Druz or others. First line therapy with cladribine was given to 159 patients (80.71%); other therapies 9.14%, while 1.1% did not receive any treatment. The median time from HCL diagnosis to treatment with 2CdA was 5.9 years. IV therapy was given to 62% of patients and 38% received it SC. Complete remission rates, progression-free survival and overall survival were not significantly different between the two schedules. In univariable analysis: Sex, ethnicity, dose, patient weight, and treatment duration (5-7 days) had no impact on outcome, but patients older >65 years had a shorter survival. Infectious complications requiring hospitalization was reported in 50.3% of all treated patients (54%, post IV and 47% post SC delivery; p=0.40). 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 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 Partial response (PR) group.

Methods: Two-hundred ninety-nine patients with CLL were retrospectively evaluated. Median follow-up was 91 months (range, 2-390). CLL diagnosis was based on IWCLL criteria. Endpoints were time to next treatment (TTT) and OS. TTT and OS curves were estimated by the Kaplan-Meier method and differences were evaluated with the log-rank test. Response to therapy was determined according to IWCLL criteria. Endpoints were time to next treatment (TTT) and OS. 124 patients were classified according to Rai clinical stage.

Results: Among patients in PR (n=66), after a median follow-up of 42.5 months (range 1-201), those patients with stage A disease at the time of response evaluation (PR Binet A) had significantly better outcome than those whose stage was Binet B/C (median survival 63 vs 43 months; p=0.047). Interestingly, when the analysis was restricted to response assessment after first line therapy (n=229), patients who achieved PR Binet A did not have significant differences. In OS compared to those patients who were in CR (median survival were 164 and 214 months respectively, p<0.001); on the contrary, patients in PR Binet B/C had a similar outcome than those who did not respond to treatment (median survival 81 and 91 months respectively; p=0.13). Similar results were observed in the outcome of patients with PR subclassified according to Rai clinical stage.

Figure 1.

Summary/Conclusions: Changes in clinical stage provide reliable information for the evaluation and stratification of patients with CLL, particularly those in the IWCLL PR category. This study supports the use of clinical stages as a complementary and simple tool to assess response in patients with CLL, both at the end and over the course of treatment.

PB1783

INCIDENCE OF THYROID Gland DISORDERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Frequency of autoimmune complications like immune 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 CLL patients were included into the study (65 male, mean age was 62±10.4). Free T3 (FT3) was within normal limits in 96 cases (96%), was low in 2 cases (2%), was high in 2 cases (2%); free T4 (FT4) was normal within normal limits in 89 cases (89%), was 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 sick 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 2 patients in ≥65 years old age group.
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 are a subset of cases with lymph node (LN) involvement by SLL in which proliferation centers (PCs) were not observed and pts in whom lymphadenopathy was < 1.5 cm showing a better prognosis.

Aims: To: a) record clinical, biological features and treatment strategy in a series of 292 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 pts diagnosed in our centers 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 containing prolymphocytes and paraimmunoblasts, surrounded by a dark background of small lymphocytes, were evaluated in hematoxylin and eosin sections and defined as pale areas.

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, 19 and 9 were classified as A, B and C stage respectively while according to Ann Arbor staging system, 74% had advanced disease stage. 11 pts presented with bulky lymphadenopathy, 11 had splenomegaly and 4 had B-symptoms. LN biopsies were performed in 37 out of 47 pts. All pts underwent bone marrow (BM) biopsy with a median BM infiltration of 45% (0-97%). PCs were identified in 19 out of 24 pts in whom data were available, while 31 pts were presented with LN > 1.5 cm as measured in CT. The estimated 10 y -OS was 80% while median TFS was 5.3 mos. Age and ECOG performance status were the only parameters that were statistically significant in terms of survival (p = 0.019 and p = 0.013 respectively).

Pts with LN>1.5cm and pts in whom there were no detectable PCs tended to have better survival. 24 pts (51%) were in need of therapy and most of them were treated with cytoreductive therapy (13 received Rituximab (R)-Chlorambucil (Chl), 3 RCVF, 2FRC, 1R Bendamustine, 2 Chl, 2R as monotherapy and 1 received corticosteroids). 23 pts were assessable for response and among them 4 entered CR, 17 PR and one had stable disease.

Summary/Conclusions: Outside the context of clinical trials SLL pts were treated mostly with lymphoma immunochemothapeutic protocols while mild treatment approaches resulted in significant responses. LN features such as size and presence of PCs tended to have prognostic significance. Further analysis in larger series of pts is on the way.

PB1785

HEMEINSIGHT TO ASSESS PATIENT REPORTED OUTCOMES OF PATIENTS AFFECTED BY CHRONIC LYMPHOCYTIC LEUKEMIA IN DAILY CLINICAL PRACTICE

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Background: Chronic lymphocytic leukaemia (CLL) is the most common leukemia in Western Countries, with a median age at diagnosis between 67 and 72 years. The therapeutic landscape of CLL is changing rapidly with the advent of small molecules acting as B-Cell Receptor (BCR) signaling inhibitors. In this setting, long term oral therapy may lead to the reduction in compliance, with a possible impact on effectiveness. Moreover, long-term follow-up may highlight complications, such as drug-related adverse events that, together with the disease itself, may impact quality of life (QoL). Patient Reported Outcomes (PROs) in daily clinical practice is a resource-intensive procedure and may be affected by low adherence, risk of recall bias and difficulties in establishing reproducible procedures. HemInsight, a project conceived in 2010 for myeloproliferative neoplasms in haematological centres in Denmark, enables patients to periodically submit PROs online to be combined to the medical records.

Aims: HemInsight was implemented at our Centre to collect PROs from CLL patients in daily practice.

Methods: HemInsight incorporated the EORTC QLQ-C30, EORTC QLQ-CLL 16, SF-36, and the eight-item Morisky Medication Adherence Scale (MMAS-8) questionnaires to collect PROs and their changes during various stages of CLL (diagnosis - progression - treatment). PRO assessments were scheduled for the patients who received regular reminders by email to complete the tasks. The following measurements will be assessed: system attraction (percentage of CLL patients adhering to the project); patient compliance in filling out questionnaires; system efficiency (number of alerts related to QoL worsening and number of questionnaires not submitted) and system effectiveness (significant differences in changes in QoL scores from diagnosis to response/relapse, changes of therapeutic approach/action following an alert, changes in adherence of therapy).

Results: At the time of the present report, 74 patients with a CLL diagnosis have been enrolled, 15 of whom were newly diagnosed. Fourteen patients underwent cytoreductive therapy and 2 are under treatment with novel oral drugs. System attraction: the study was proposed to 91 consecutive patients, have better survival. 24 pts (51%) were in need of therapy and most of them were treated with cytoreductive therapy (13 received Rituximab (R)-Chlorambucil (Chl), 3 RCVF, 2FRC, 1R Bendamustine, 2 Chl, 2R as monotherapy and 1 received corticosteroids). 23 pts were assessable for response and among them 4 entered CR, 17 PR and one had stable disease.

Summary/Conclusions: Outside the context of clinical trials SLL pts were treated mostly with lymphoma immunochemothapeutic protocols while mild treatment approaches resulted in significant responses. LN features such as size and presence of PCs tended to have prognostic significance. Further analysis in larger series of pts is on the way.

Table 1.

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In conclusion, HemInsight is a useful tool for QoL evaluation in CLL patients. Provisional data suggest a higher compliance of those patients who feel that they need a closer contact with the clinician, both for individual disposition or disease status.
Background: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia in adults in the USA, accounting for 20% to 30% of all leukemia cases. CLL affects mainly elderly patients, with a median age at the time of diagnosis reported to be 71 years. Although CLL is not curable, disease symptoms and progression may generally be controlled with adequate pharmacologic treatments. Bendamustine-based regimens have long time been used in the management of CLL patients but few studies have analyzed the comorbidity- and/or adverse event (CAE)-related healthcare costs in elderly patients receiving these regimens in a real-world setting.

Aims: To describe all-cause and CAE-related healthcare costs of elderly patients with CLL treated with a bendamustine-based regimen in second or later lines of therapy in a real-world setting.

Methods: A retrospective cross-sectional cohort study design was used. Adult patients who received a bendamustine-based regimen in second or later lines of therapy on or after January 2010 were identified from the Medicare Limited Data Set (LDS) 5% Standard Analytic Files (data availability: 1999–2014). The index date was defined as the initiation date for the first of the studied bendamustine-based regimens. Selected patients were required to be continuously enrolled in their Medicare plan for ≥6 months before and ≥3 months after the index date – unless the patient died during the first 3 months after the index date. Patient cohorts were determined based on the treatment initiated on the index date (index treatment): the two most prevalent bendamustine-based regimens were analyzed, i.e., (1) bendamustine and rituximab in combination (BR cohort) and (2) bendamustine monotherapy (bendamustine cohort). Healthcare costs, including inpatient, emergency room, outpatients and CLL-drug costs, incurred while treated with the index treatment were described for each cohort. For each medical cost component, all-cause and CAE-related costs were summarized. Healthcare costs were adjusted for inflation (2016 USD) and reported per-patient-per-month (PPPM).

Results: A total of 275 patients were included in the BR cohort and a total of 100 patients in the bendamustine cohort. Most patients (61.8% in the BR cohort and 65.0% in the bendamustine cohort) were male and the mean age was approximately 75 years old. During the 6 months prior to the index date, patients in the BR and bendamustine cohorts were similar in terms of comorbidity profile; mean Charlson comorbidity index was 3.53 in the BR cohort versus 3.62 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 66.4% 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, 2017) for treatment initiation, assessment of residual disease and minimal residual disease (MRD). Induction chemotherapy was conducted under the following programs: RB, FC, RFI, 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 in 144 (72.7%) patients: Rituximab 500 mg/m² intravenously every 8 weeks (n=116) for 2 years, Ibrutinib 420 mg, orally, daily (n=28) continuously. The remaining patients (n=54) were under dynamic observation without therapy.

Results: The increasing depth of response (from partial (PR) to complete remission (CR)) was observed only in group of patients receiving MT – 10.4% (15/144) (p=0.013). The frequency of increase the depth of remission in the patients treated with MT ofibrutinib was 28.6% (8/28), MT of rituximab – 6.0% (7/114) (p=0.0005). The medians of PFS and duration of response were a longer in the patients with MT versus in the patients without MT: PFS – 48 months and 37 months, respectively (p=0.03); duration of response – 44.0 months and 25.5 months, respectively (p=0.0006). The median of duration of response in the patients with MT of rituximab was 28.6 months, while in the patients without MT – 25.5 month (p=0.004). The frequency of relapses in the group of patients with MT was 39.6% (57/144), in the group of patients without MT – 66.7% (36/54) (p=0.0007). Recurrence of the disease occurred more frequently in the group of patients treated with MT of Rituximab, compared with Ibrutinib: 45.7% (53/116) and 14.3% (4/28), respectively (p=0.002). The median duration of observation in the group with rituximab was 22 months, while in the group with Ibrutinib – 11 months. MRD was not detected after 6-12 months of MT in 23.5% (12/51) had previously MRD-positive patients. Among patients with MRD-negative CR relapse is less common than in patients with MRD-positive CR – 20.0% (4/20) versus 62.5% (10/16), respectively (p=0.009).

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 LST SYSTEM
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Background: The BD OneFlow solution for diagnostic screening of chronic lymphoproliferative disorders (CLPDs) includes a standardized flow cytometry approach based on the EuroFlow (EF) Consortium liquid reagent system. The BD OneFlow solution enables reproducible identification and discrimination of normal from aberrant mature cell populations by combining standardized assays, set up regents, 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 leucocytes. The FCS files from evaluable specimens of the original LST clinical trial were retrospectively analyzed using BD OneFlow LST template v1.0.

Aims: The object of this study was to reprocess the FCS files from all the evaluable specimens previously collected using LST template v1.0 in the original clinical study to demonstrate equivalency between the investigational BD OneFlow LST system and the comparator EF liquid reagent system on a BD FACSCanto II flow cytometer with the 4-2H-2V CE-IVD configuration and LST template v1.0.

Methods: The FCS files using LST v1.0 template from the original clinical study included de-identified remnant peripheral blood (n=123), bone marrow (n=53), and lymph node (n=31) specimens from patients and healthy donors. Specimens...
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 system and comparator EF liquid reagent within 24 hours of collection and were acquired within 60 minutes of staining. In the current study, analyses were performed on a BD FACSCanto II instrument using LST v2.0 template. For follow-up, endpoint results for T-cell, B-cell, and NK-cell lineages 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% agreement (95% CI: 98.6%) and delineating patients into normal (no follow-up) or follow-up, and 100% overall agreement in identifying B-, T-, and NK-cell lineages (95% CI: 98.6%). There was 100% positive agreement and 100% negative agreement between BD OneFlow and EF for follow-up vs no-follow-up (normal) and for all cell lineages from specimens that required follow-up. Furthermore, compared to the BD OneFlow system, the BD OneFlow LST system met the acceptance criteria for the quantitation of normal (207 of 207) overall agreement (lower 95% CI: 98.6%) in delineating patients into normal (no follow-up) or follow-up.

Summary/Conclusions: The multisite performance evaluation of the BD OneFlow LST system and the comparator EF liquid reagent system was concordant in identifying abnormal from normal mature populations in patients with CLPDs. BD OneFlow LST is fit for in vitro Diagnostic Use. CE Marked to the European in Vitro Diagnostic Medical Device Directive 98/79/EC. 23-19566-00.

PB1789

IMMUNOGLOBULIN HEAVY/LIGHT CHAIN ASSAY DETECT IMMUNE DYSREGULATION IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA


Background: Chronic lymphocytic Leukemia (CLL) is frequently accompanied by immune dysregulation. Hypogammaglobulinemia is the most important associated immune defect and all three classes of immunoglobulins (IgG, IgA, and IgM) 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. Serum samples were analyzed for levels of: IgG1, IgG2, IgG3, IgG4, IgA kappa, IgA lambda, IgM kappa, IgM lambda and Free light chain: kappa (K) and lambda (L), ratio of K/L and calculation of ratios of monoclonal/polyclonal immunoglobulin (HLC ratio).

Results: The total cohort consisted of 126 treatment-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 levels of immunoglobulin kappa (p-value of 0.001, 0.005 and 0.001 respectively). Abnormal IgG-lambda values were evident in 15 patients (10%) and associated with more pronounced leucocytosis (p=0.05), higher B2mg levels (p=0.022) and the presence of 17p deletion (p=0.001). Juno IgM lambda were abnormal in 35% and 56% of cases respectively compared to 8% and 9% in the controls. For IgG subclasses: both IgG2 and IgG4 levels were significantly lower in CLL patients than in controls (p=0.001 and 0.001 respectively). In addition, IgG2 and IgG4 were also confirmed to be significantly lower in CLL patients than in controls (p>0.05). For IgG2 levels, we observed a significant increase in the Binet stage and elevated LDH levels. Abnormal lambda FLC was observed in 26% of patients and only in one (4%) healthy control. Abnormal free light chain ratio (FLC) was present in 39 patients (32%), with a mean value of 4.22 (0.11-62.15) while only 1% (4%) was observed in healthy controls (mean value of 1.76 (0.25-2.3)) and was also associated with more advanced Binet stage and elevated LDH (p=0.003).

Summary/Conclusions: CLL cells may produce light chains, or as shown here abnormal intact immunoglobulins (heavy and light chains), HLC levels were associated with advanced stage and adverse prognostic parameters. These findings lend support for the considerable potential of the HLC assay in the evaluation of clinical status in patients with CLL.

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 leukaemia (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 IFNα or 2-CdA and to estimate the relationship with blood count indexes in HCL pts.

Methods: The study group consisted of 26 primary pts with the classic variant of HCL (median age - 47 years). A control group consisted of 12 healthy persons (median age - 50 years). The concentration of cytokines was measured using a validated commercial ELISA kits.

Results: Median of TNFα content in serum of HCL pts before treatment was substantially lower (3.57 pg/ml) than in healthy persons (8.56 pg/ml; p=0.275), however levels of IL-6, IFNα or 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-}
on distribution of response in clinical trials. Data on disease progression and mortality was provided by the treating oncologist/hematologist. PFS and OS were compared using univariate and multivariate Cox proportional analyses between the CR and non-CR cohorts (OS multivariate analyses were not conducted due to the small number of events). An additional analysis was conducted to examine the benefits of achieving MRD- versus not achieving MRD- among patients who achieved CR or PR.

**Results:** Data was collected on 330 CLL patients, including 179 patients in the CR cohort and 151 patients in the non-CR cohort (120 patients with PR, 25 with SD, and 6 with PD). Most patients were male, in their early sixties, and had an ECOG status of 0/1 at the time of initiating first-line therapy. The median observation period was approximately 30 months. There were 43 (24%) patients in the CR cohort and 75 (50%) patients in the non-CR cohort who progressed/died (Table 1). Patients in the non-CR cohort had an >2-fold higher hazard of progression/death (adjusted hazard ratio [HR]=2.30, p<0.05) and death (unadjusted HR=2.61, p<0.05) compared to patients in the CR cohort. Among patients who achieved CR or PR, 84 patients achieved MRD- and 62 patients did not; 14 (17%) patients who achieved MRD- and 27 (44%) patients who did not achieve MRD- progressed/died. Patients who did not achieve MRD- had an over three-fold higher hazard of progression/death compared to patients who achieved MRD- (adjusted HR=3.75, p<0.05). No death events were observed among patients who achieved MRD- while 4 (6%) events were observed among those who did not achieve MRD-.

**Table 1.**

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<thead>
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<th>Event</th>
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<tr>
<td>Death</td>
<td>35</td>
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<tr>
<td>Progression</td>
<td>23</td>
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**Summary/Conclusions:** Findings from this real-world study suggest that achieving CR is associated with improved PFS and OS compared to patients who do not achieve CR. Furthermore, significantly better outcomes were observed among patients who achieved MRD- compared to those who did not achieve MRD- but still achieved CR or PR. This suggests that deep response may be an important clinical parameter to consider in the treatment of CLL.

**PB1792**

**ANTI-CD ANTIBODY MICROARRAY FOR MORPHOLOGY EXAMINATION OF CIRCULATING LEUKEMIA AND LYMPHOMA CELLS**

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**Background:** Matching the morphology with immunophenotype for individual leukocytes is a major issue in diagnostics 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 leukemias and lymphomas are morphologically identical to the same cells in blood smears.

**Methods:** Anti-CD antibodies were immobilised on plastic coverslips in spots 2 mm in diameter. In order to study the peripheral blood mononuclear cells (PBMC) for the monoclonal 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 cytostainer and stained after May–Grünewald-Giemsa for morphology examination. Using this technique we have studied the PBMC from 56 healthy donors and 77 patients with leukemias and lymphomas: chronic lymphocytic leukemia (CLL, 37 patients), hairy cell leukemia (HCL, 22 patients), splenic marginal zone lymphoma (SMZL, 7 patients), mantle cell leukemia (MCL, 2 patients), follicular lymphoma (FL, 1 patient), 5 patients with multiple myeloma (MM), 2 patients with large granular lymphocytic (LGL) leukemia and one patient with acute myeloid leukemia (AML12).

**Results:** None specific cell binding both inside and outside the spots is below 5%. Due to the non-mixing incubation the density of the cells bound to an anti-CD antibody permits to determine the proportions of cells positive for the corresponding CD antigen with high correlation with flow cytometry. The patterns of the binding densities of the anti-CD-captured PBMC for CLL, HCL and SMZL patients clearly differ both from those for normal PBMC and from each other and agree well with the reported immunophenotypes of corresponding neoplastic cells. Both normal and pathologic microarray-bound PBMC after the proprietary drying procedure are morphologically identical to the same cells in a smear. In cases when pathologic cells are morphologically and/or cytogenetically distinct, the anti-CD antibody microarray permits to determine their percentage and immunophenotype by analysing the relative amount of these cells captured by the antibodies against all the CD antigens. The results of such analysis of neo-plastic PBMC for the patients with leukemias and lymphomas agree with flow cytometry results for the same patients including CLL, HCL, CD2 and CD11c in CLL. CD56 in MM. The amount of hairy cells determined morphologically on the microarray varied from 20 to 97% of all anti-CD19-captured cells and 2 to 80% of all lymphocytes and was in good agreement with the percentages of cells with CD19/CD103 and CD19/CD11c coexpression determined by the peripheral blood of the same patients by flow cytometry.

**Summary/Conclusions:** The microarray works as a “sorted smear” with cells positive for certain surface CD antigens localised in a predetermined area and permitting to apply any standard smear-oriented technique to the microarray-captured cells. Combined analysis of the pathologic cells’ immunophenotype, morphologic and cytogenetic data which are then applied to patient’s history allows to create a decision tree facilitating morphologic and cytochemistry on the microarray - permits to arrive at preliminary diagnosis and can be used in cases of any controversies between morphology, cytochemistry and immunophenotyping. The work is partially supported by 16-34-0130 and 16-04-00282 grants from RFBR.

**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 time to first treatment (TTFT) and overall survival (OS) of chronic lymphocytic leukemia (CLL) patients with chronic lymphocytic leukemia (CLL-IPI) developed by The International CLL-IPI working group was found to predict OS and TTFT, while the rest of two scores - progression-risk score (PRS) and MD Anderson Cancer Center Score 2011 (MDACC 2011) have been developed for prediction of TTFT in early stage CLL patients.

**Aims:** The aim of this study was to compare CLL-IPI, PRS and MDACC 2011 prognostic scores based on their impact on TTFT, treatment response (TR), progression-free survival (PFS) and OS of 54 treated CLL patients.

**Methods:** We retrospectively analyzed data from 54 consecutive CLL patients diagnosed and treated at Clinic for Hematology, Clinical Center Score 2011 (MDACC 2011) have been developed for prediction of TTFT 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 Oncology 2011 for CLL-IPI; de Vries et al, 2013, 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 fludarabin-based chemotherapy, 45 (83%) in the first treatment line. Overall response rate to the first line therapy was 81%, equally distributed on complete and partial responses. Most of the patients (42, 78%) relapsed during the follow up. At the time of the last follow up 14 (26%) patients were still alive, 35 (65%) were dead, and 5 (9%) were lost to follow up. Median overall survival was 76 months. Lower score values for all the three scoring models predicted better TTFT, 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

Rearrangements of the IGHV genes were performed in 100 CLL cases. The previously launched BD OneFlow LST (Lymphocyte Screening Tube) is intended for flow-cytometric immunophenotyping of normal (no follow-up required) and aberrant (follow-up required) mature lymphocyte populations of B, T, and NK lineages in specimens from patients with hematological disorders. The BD OneFlow B-CLPD T1 is being developed to work in conjunction with BD OneFlow LST for the immunophenotyping of B cells and distinguishing chronic lymphocytic leukemia (CLL) from other B-CLPDs such as atypical CLL, follicular cell lymphoma, mantle cell lymphoma, etc.

Methods: De-identified remnant peripheral blood (PB) (n=70) and bone marrow (BM) (n=30) patient specimens were collected in EDTA or heparin anticoagu-

atants at four external study sites and tested within 26 hours of draw. Informed consent was not required in this clinical study. Specimens were stained with BD OneFlow LST in combination with OneFlow B-CLPD T1 tubes and comparator EF liquid reagents. Acquisition and analysis were performed on a BD FACSCanto II instrument using BD OneFlow LST and B-CLPD T1 templates in BD FACSDiva software v8.0.1. Categorization of samples with abnormal B-cell populations into CLL (typical) or other B-CLPDs, overall agreement, negative agreement, and positive agreement, along with their one-sided lower 95% confidence limits, were calculated.

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 based on the relative fluorescence intensity of CD45+CD19+ abnormal cell populations, overall agreement of 97.4% was calculated.

Summary/Conclusions: The multisite performance evaluation between the BD OneFlow LST and the comparator EF liquid reagent system on the BD FACSCanto II flow cytometer using BD FACSDiva software demonstrated 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 FACSCanto II flow cytometer using BD FACSDiva software.

References:
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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 overlaying configurations of IGHV gene usage, IGHV mutational status and cytogenetic alterations. These included a group which mainly consisted of clinical advanced stage CLL with a skewed but different IGHV-associated IGHV gene repertoire (VH1-69 associated with HD3 gene and HJ6 gene) and second group enhanced in clones expressing specific IGHV subgroups (VH3-23 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 distinct geographic areas and microenvironments are required to validate our findings and discard or confirm the potential role of some antigen-binding BCR specificities contributing to clonal evolution.
Background: Constitutive activation of B-cell receptor signalling appears to be essential for the proliferation of malignant B cells. Bruton’s tyrosine kinase (BTK) has been identified as an essential component of the B-cell receptor signalling pathway. Ibrutinib is an orally administered BTK inhibitor that antagonises B cell receptor, chemokine & integrin mediated signalling.

Aims: We report our experience of using ibrutinib to treat relapsed/refractory B-cell chronic lymphocytic leukaemia (B-CLL) and mantle cell lymphoma (MCL) in a busy U.K. District General Hospital (DGH) serving a population of 600,000

Methods: 26 patients were commenced on ibrutinib for relapsed/refractory B-CLL or MCL between August 2014 & December 2016. 16 patients had B-CLL and 10 patients had MCL. Patients with B-CLL were commenced on 420 mg daily; those with MCL received 540 mg 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 time of ibrutinib was 6.7 months. At the last available visit or last dose received. The average number of co-morbidities in both groups was similar: 1.4 in B-CLL and 1.5 in MCL. After May 2015 all patients received aciclovir and co-trimoxazole prophylaxis. Response to ibrutinib was assessed by clinical examination and blood results; imaging and bone marrow examination were conducted at the clinician’s discretion.

Results: The median follow up was 15.5 months for B-CLL patients and 8 months for MCL patients. The median survival of all patients who did not receive anti-viral and pneumocystis prophylaxis was 5 months and the median survival for those who did receive prophylaxis was not reached (p = 0.0001). The median survival of patients who received ibrutinib as a 1st treatment was 17 months; the median survival in those who had received just one prior line of treatment was not reached (p = 0.0085). In the B-CLL cohort there was no difference in survival between those who with and without 1p / p53 deletion. 11/26 patients experienced side effects: 8 had grade 1 and 2 side effects (diarrhoea, drug rash, cardiac arrhythmias) which were easily controlled. 3 patients had grade 4 side effects (1 severe arthropathy, 2 intracranial haemorrhage - one of which was fatal). 4 of the 16 (25%) with B-CLL and 5 of the 10 (50%) with MCL died during the period of follow-up. Causes of death were: intra-cerebral haemorrhage (1), unrelated cancer (1), disease progression (2), disease progression+sepsis (2), sepsis alone (3). Of the remaining 17 patients, 14 continue to receive ibrutinib, 2 (B-CLL) were switched to idelalisib+Ibrutinib (for grade 4 toxicity) & 1 went on to have an allogeneic transplant (MCL).

Summary/Conclusions: Though our cohort of patients is small, our experience shows that the use of prophylaxis with co-trimoxazole and aciclovir is associated with improved overall survival. Moreover, patients who received fewer lines of prior treatment had a better survival. Patients with 1p/p53 deleted B-CLL responded as well as those without a deletion. Ibrutinib is a very effective therapeutic option in patients with relapsed CLL and MCL.

PB1799

Richter syndrome: Serbian lymphoma group experience

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Background: Richter syndrome (RS) represents transformation of chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL) into more aggressive B-cell lymphoproliferative disorder, most commonly, diffuse large B cell lymphoma (DLBCL), very rarely classical Hodgkin lymphoma (HL). In some point of disease course, 2-10% of all CLL/SLL population develop RS, usually exhibiting chemo-resistance 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 overall survival.

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 Hospital Center Zemun; Clinic of Hematology, Medical Military Academy; and Clinic for Hematology, Clinical Center Kragujevac. In all of RS patients, diagnosis was established after histopathological and immunohistochemistry analysis of biotyped tissue (lymph node, bone marrow, Waldeyer’s ring, maxillary sinus, spleen or liver).

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. On presentation, clinical 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 4 (11%) patients were treated with RS prophylaxis and 5 patients (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. Prior to transformation, 18 patients received second line chemotherapy, 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 0.951-0.995; 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).
mainly to patients with low IgM concentration in the blood serum and at more advanced clinical stages of the disease. In this case the occurrence of previous complications (before ibrutinib administration) is also relevant. We are aware of the limitations of our work related to the small number of patients. Yet, even at this stage, it is possible to select CLL patients with increased risk of such usually life-threatening complications.

**PB1801**

**MONOCLONAL B-CELL LYMPHOCYTOSIS AND PROSTATE CANCER: AN UNEXPECTED, POSSIBLE ASSOCIATION**

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**Background:** Monoclonal B-cell lymphocytosis (MBL) is a recently recognized entity characterized by the presence, in the peripheral blood, of a monoclonal B-cell population lower than 5000/µl, in the absence of any type of clinical features. MBL clones may have: a) chronic lymphocytic leukemia (CLL-like) phenotype (CD5+, CD19+, CD23+, CD20 dim); b) atypical CLL phenotype (CD5+, CD19+, CD23- or CD20 bright); c) non-CLL phenotype (CD5-). MBL can be also distinguished in “low-count” (<500/µl) and “high-count” (>500/µl) subtypes. High-count MBL frequently shows typical CLL phenotypic/genetic features and require adequate follow-up in order to detect their possible evolution into symptomatic CLL. MBL showing a clonal B-cell count higher than 1000-1500/µl are usually defined “clinical” MBL. Using highly sensitive (i.e. >6 colors and >500000 events) flow cytometry approaches, CLL-like MBL clones have been found at a frequency of 7-12% in healthy subjects, showing, however, very low median counts of clonal B-cell (10-170/µl), with only 0.14% being clinical MBL. Though several studies have described the association between CLL and various types of neoplastic disorders, only few data exist about the risk of non-hematologic cancer in individuals with MBL, in particular, no association between MBL and prostate cancer (PC) has been so far reported.

**Aims:** To study prospectively the frequency of CLL-like MBL clones in patients affected by PC compared to healthy males of the same ages, after our previous observational study of an apparently increased MBL incidence at baseline in a cohort of patients with PC finally observed to detect lymphocyte abnormalities possibly induced by radiotherapy (RT).

**Methods:** We enrolled 34 consecutive patients affected by PC (mean age 74 years, range 58-91), naive for chemotherapy (sixteen previously treated with hormone-therapy). All patients were planned to receive whole-pelvis RT with radical (n. 23) or salvage (n. 11) intent. Fifty-four healthy males (mean age 71 years, range 58-87) represented the control group. Immunophenotypic analysis of peripheral lymphocytes before RT was performed by BD FacsCanto II flow cytometer, using a 5-6 colors approach and the following antibody combinations: CD19 FITC/CD5 PE/CD45 PerCP/CD20 PE-Cy7/CD23 APC, Kappa FITC/Lambda PE/CD19 PerCP-Cy5.5/CD20 PE-Cy7/CD5 APC/CD45 APC-Cy7. For each sample, 100000 events were collected. CD45+ lymphocytes were gated on CD45 vs SSC dot plot, then B cells were isolated by gating on CD19 and CD19+ cells were interrogated for intensity of CD20. Finally, CD19+ CD5+ CD20dim selected population was analyzed for light chain clonality and CD23 expression.

**Results:** Median (range) absolute counts of white blood cells (WBC), total lymphocytes and B-cells, as well as absolute single values of MBL clones are reported in Table 1. In PC patients we found 3 MBL (8.8%), two of which were “high count/clinical” MBL (5.8%). In contrast, in healthy subject group, only one “low count” MBL (1.8%) was detected, showing a very small clone (8 cells/µl). Such a difference was not statistically significant (p=0.2).

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<td><strong>Prostate cancer (n=34)</strong></td>
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<td>Median WBC (range)</td>
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<td>MBL n. (%)</td>
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**Summary/Conclusions:** The preliminary results of our prospective study, performed using a routine, not highly sensitive flow cytometry approach, highlight a possible association between (clinical?) MBL and PC, never described before and probably warranting further investigation in a larger number of patients.

**PB1800**

**INFECTIOUS COMPlications in Patients with Chronic Lymphocytic Leukemia treated with Ibrutinib**

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**Background:** Chronic lymphocytic leukaemia (CLL) is characterised by frequent co-existent infectious complications. They stem from, among other things, hypogammaglobulinaemia, which is connected with CLL, and correlates with the disease duration and severity, as well as T-lymphocyte function disorders. The application of innovative therapies (chemoimmunotherapy) on the one hand facilitates considerable improvements in treatment outcomes and on the other hand it increases the risk of life-threatening infectious complications. The introduction of a new drug, ibrutinib (Bruton’s kinase inhibitor), has created a unique opportunity for CLL patients, especially those with prognostically unfavourable genetic aberrations (del17p), or in the case of whom previous chemotherapies have failed to give satisfying results. Previous observations indicate the risk of side effects (e.g. bleeding, infectious complications, heart rhythm disorders) which might sometimes limit the applicability of ibrutinib in some CLL patients.

**Aims:** The aim of this paper was to evaluate the risk of infectious complications in persons with CLL, and to determine potential correlations between possible infectious complications and selected clinical, morphological and biochemical parameters.

**Methods:** The study comprised 43 CLL patients aged 48-82 years (average age 67 years), 18 women and 25 men. At the beginning of the ibrutinib therapy the patient’s disease was at the 2-4 clinical stage, according to Rai et al. Usually they were individuals who had received a couple of previous chemotherapies (from 1 to 7) which contained, inter alia, purine analogues, and the monoclonal phenotype (CD5+, CD19+, CD23-, CD20 bright); c) non-CLL phenotype (CD5+). MBL can be usually defined “clinical” MBL. Using highly sensitive (>6 colors and >60000 events) flow cytometry approaches, CLL-like MBL clones have been found at a frequency of 7-12% in healthy subjects, showing, however, very low median counts of clonal B-cell (10-170/µl), with only 0.14% being clinical MBL.
**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-ABL1 fusion oncogene. This cell line is the K562 BCR-ABL1-positive human erythroleukemia cell line derived from a female patient with CML in blast phase and representing an important tool for the studies of malignant hematopoiesis in last decades. K562 karyotype was described several times, detailed genomics 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 K562 and 1000 genomes projects and our internal exome database were filtered out and the subsequent analysis was focused on putative protein damaging variants with the frequency in the database from NHLBI GO exome sequencing project less or equal to 0.01. We used different bioinformatic tools for variant effect prediction (e.g. Polymorph-2, SIFT, IntOGen). Mutations were confirmed using Sanger sequencing. FISH was performed using commercially available probes (Vysis, Abbott, USA), that identifies BCR-ABL1 fusions.

**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).

**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:** Our study indicated that leukemia cells have acquired resistance through somatic mutation or exon 4 deletion in the BCR-ABL1 gene. MEK inhibitors (MEKi) are suggested that 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-ABL1i) using an automated cartridge-based Genexpert system (Cepheid, Sunnyvale, CA, USA).

**Results:** On analyzing 37 adult patients, all had residual Philadelphia (Ph) chromosome (100%). Classic Ph fusion pattern was seen in 33 (89%), derivative chromosome 9 (der(9)) deletions in 25 (67.5%) and supernumerary Ph chromosomes in 11 (30%) patients. Coexistence of classical fusion and der(9) deletions were seen in 21 patients (57%), whereas 8 patients (22%) had a mutual existence of classical fusion, der(9) deletions and supernumerary Ph chromosomes. None had Ph amplification. Figure 1 demonstrates the I-FISH patterns seen in a 43-year-old male diagnosed with CML-CP and had progressed to blast crisis at his 72nd month of imatinib therapy. In this Figure red, yellow and white arrows indicate blast cells without Ph chromosome, Ph+ blast with a loss of residual ABL1 on der(9)classical and random signal overlap, respectively. A mean (± S.D) of 29% (± 30) and 18% (± 17) der(9) deleted cells were seen amongst patients with b2a2 and b3a2 BCR-ABL1 transcript types, respectively and this difference was statistically significant (p<0.008). There was also a significant difference in the disease transformation status according to the percentage of der(9) deleted cells (p<0.03). In this regard, patients with progressive disease (accelerated phase/blast crisis progression) had a mean (± S.D) of 47% (± 35) der(9) deleted cells in comparison to 19% (± 20) such cells in patients without disease transformation. In addition, patients with Ph duplication/triplication had a mean (± S.D) BCR-ABL1i levels of 49.478% (± 40.184), in comparison to BCR-ABL1i 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.

**Figure 1.**

**PB1806**

**ARE YOU ACTUALLY SUSPECTING A CHRONIC MYELOID LEUKEMIA WHEN ORDERING A BCR/ABL RT-PCR?**

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**Background:** Chronic myeloid leukaemia (CML) is a myeloproliferative neoplasm (MPN). It is characterized by a reciprocal t(9:22)(q34;q11.2) resulting in the fusion oncogene BCR/ABL in a hemopoietic stem cell. Clinical features are absent in nearly 20-40% of patients at diagnosis time. Hence, laboratory suspicion is crucial. Peripheral blood shows leukocytosis with left shift and “myelocyte bulge”, absolute eosinophilia, and absolute basophilia invariably present1-3. The demonstration of the Philadelphia (Ph) chromosome with cytogenetic analysis, or BCR/ABL fusion gene by qRT-PCR will confirm the diagnosis (typical CML).

**Aims:** In order to gain accuracy when BCR/ABL PCR is ordered, we review myeloproliferative 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 specialities (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 >7.7x10⁹/L, and basophilia was defined as absolute basophil count of >0.2x10⁹/L. A total of 299 requests for PCR of BCR-ABL were reviewed by laboratory hematology staff. We were performing the classification of Hematologic 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, monocytosis, splenomegaly or combined). Among 24 positive cases 100% presented basophilia at diagnostic time and 91.66% (22/24) presented basophilia and neutrophilia. Two cases without neutrophilia at diagnosis were CML with extreme trombocytosis. We found 33 cases with basophilia among 235 patients. 24 cases (72.73%) were diagnosed of CML and 9(27.27%) resulted in other MPN Ph- or unclassifiable MPS/MDS neoplasm. Our results show that when CML is suspected, basophilia>0.3 x10⁹/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 x10⁹/L, further investigation should be performed in order to diagnose a MPN Ph- or MDS/MPN. Even basophilia is well established as clearly universal in CML 1,3,4, this study reveals it is not always pursue enough, when clinicians ask for a molecular study.

**Figure 1.**

**PB1807**

**BCR-ABL DEL C.1086-1270 (P.R362FS*21) AND TKI RESISTANCE IN CML PATIENTS FROM RUSSIAN FEDERATION**

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Background: Data concerning the impact of BCR-ABL del. c.1086-1270 on TKI resistance in CML is still controversial. This mutation was first described by Curvo et al. (2008) and was thought to confer TKI resistance. However computer modeling performed by Meggynesi N. et al. (2012) revealed disruption of ATP binding site in mutated tyrosine kinase therefore abrogating enzymatic activity. Nevertheless pathogenic effect of BCR-ABL p.R362fs*21 could be attributed to the formation of heterodimer with “wild type” Bcr-Abl p210 as described by Poulakakos P. 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 expressed on the same BCR-ABL 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 p210 transcript.

PB1808
PEROXIREDOXIN II ACTIVITY HAS IMPORTANT ROLES TO CONTROL ABL TYROSINE KINASE ACTIVITY IN STIS TREATED CML PATIENTS AND ITS POTENTIAL APPLICATION IN IMATINIB RESISTANCE

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Background: Therapies targeting the redox environment such as over-expression of antioxidants or antioxidant treatment, could inhibit tumor cell growth even resistant cells. Bcr-Abl oncoproteins is known to induce high levels of intracellular ROS which may further induce genomic instability with malignant transformation and even cell death. Variable expression of antioxidant enzymes in leukemia, with limited studies with variable results so far. Altered redox biology in leukemia also has implications for therapeutics.

Aims: We investigated the roles of PRX II in CML primary cells at diagnosis and remission during signal transduction inhibitor (STIs), and tested the same roles in Ph+ cell lines.

Methods: Three BCR-ABL1 positive cell lines with different resistance to TKI and generating IM-resistant K562 cells by chronic exposure of increasing concentrations of IM were compared with cell growth by MTT assay. BCR/ABL expression by western blot analysis, changes of intracellular ROS level and antioxidant enzymes such as peroxiredoxin (Prx) 1, 2, 3 using immunoblot assay according to different concentrations of IM between 0 to 10 μM in time dependent manner (24 hours/48 hours). We also repeatedly investigated the effects of IM therapy using PRXII overexpressed K562 cells by transfection.

Results: Three BCR-ABL1 positive cell lines showed significant change in cell viability. Intracellular ROS level, eradication of BCR/ABL oncogene and levels of Prx2 during IM treatment with different response each other in degree and pattern by IM exposure. The levels of BCR-ABL1 oncogene were slightly decreased in Prx2 overexpressed K562 cells. Moreover, Prx2 overexpressed K562 cells showed lowered down-regulation of Bcr-Abl oncoprotein by IM treatment.

Summary/Conclusions: Our findings may contributes to find a new pathway on which TKIs are working besides the mechanisms of ATP binding competitively, blocking the binding of ABL-kinase and substrate resulting apoptosis of Ph+ cells. In addition develop the new strategies to overcome the situation of the Imatinib resistance can be solved BCR-ABL positive disease in the future. The importance of the roles of ROS and its PRX II, antioxidant enzymes in CML is further established by our work.

PB1809
FUNCTIONAL CHARACTERISTICS OF ERYTHROID PROGENITOR CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH IMATINIB AND NILOTINIB

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Background: It is believed that chronic myeloid leukemia arises as a result of myeloid progenitor cell malignancy. There are changing of proliferative activity in granulopoietic lineages 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 minterleukin-6 and interleukin-9. Cultivation was provided 14 days, then counted the number of erythroid colonies and provided their morphological studies.

Results: The results showed that the increase of erythroid progenitor cells proliferation rates and the reduction of differentiation rates as a result of the parallel cultivation of patients’ bone marrow cells in vitro 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, allowing cytokines and growth factors of mouse body affect human mononuclear cells. However, correlation 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 can be 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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cases) of a small chromosome denominated Philadelphia chromosome, coming from the reciprocal translocation between the chromosomes 9 and 22. Depend on where the break-point occurs, different isoforms of the fusion gene BCR-ABL may appear. For the diagnosis of CML, detection of BCR-ABL rearrangement is crucial; and molecular biology techniques, such as RT-PCR, may be the only data at that point, but most current RT-PCR methods for detecting BCR-ABL are designed and optimized for detecting the major forms (e14a2 and e13a2) without distinguishing between them. Characterization of the transcript is not necessary for the diagnosis but permits follow-up at the molecular level and differentiate between different BCR-ABL isoforms at the time of the CML diagnosis could be taken into account in future studies to investigate its role in the evolution of CML.

Aims: To develop a new multiplex RT-PCR method coupled to fragment analysis by capillary electrophoresis to identify different BCR-ABL isoforms: e13a3, e19a2, e19a3, e6a2, e1a3, e13a2, e14a2, e1a2 and e8a1.

Methods: 34 CML patients BCR-ABL positive by qRT-PCR and 1 negative control were analyzed for BCR-ABL isoforms. In this study, total nanograms of total RNA from leucocytes were used for retro-transcription (SuperScript® IV). Subsequently, Multiplex PCR reactions were assessed using primers described by Burmester in 2008 [ABL-3 primer labeled with carboxyferrescin (FAM)]. G6PD was chosen as endogenous gene control using G6PD-Human GenBank accession number M31381. Multiplex PCR reactions were analyzed using fluorophore-labeled primers and the capillary electrophoresis system ABI3130XL analyzer, using ILS600 as marker.

Results: BCR-ABL fusion RNAs were detected in all patients (34/34), on the other hand we did not detect BCR-ABL on the negative control. The main isoform identified was e14a2 (detected in 22 out of 34 patients, 64.7%). Twelve patients were positive for e13a2 BCR-ABL isoform (35.3%). Interestingly we identified 7 patients (20.5%) with co-expression of e14a2 and e13a2 isoforms, being in all these cases the e14a2 isoform mainly expressed.

Summary/Conclusions: RT-PCR combined with capillary electrophoresis is a very sensitive and accurate technique for the detection of different isoforms of BCR-ABL and may be included as a BCR-ABL first screening. Quantification with qRT-PCR might only be done in positive samples. Unfortunately we could not detect any isoform besides the majority ones, due to the size of our cohort. Finally, our study validates previous studies on the main BCR-ABL isoforms (e14a2 and e13a2) percentage detected in CML patients.

PB1811

Abstract withdrawn.

PB1812

PDGF AND BDNF PLASMA LEVELS IN CML PATIENTS BEFORE AND AFTER INITIATION OF TKI THERAPY

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Background: Chronic myeloid leukemia (CML) is a malignant myeloproliferative neoplasm, which is characterized by t(9;22)(q34.1;q11.21) translocation, also known as the Philadelphia chromosome (Ph). The resulting fusion gene BCR-ABL encodes a constitutively active tyrosine kinase that dictates the pathophysiology of CML. Tyrosine kinase inhibitors (TKIs) have been shown to efficiently inhibit not only the Bcr-Abl kinase, but also act on other cell surface tyrosine kinase receptors, such as the platelet-derived growth factor receptor (PDGFR). Similar receptors are vital in neutrophin-mediated signaling pathways, for example TrkB receptor for brain-derived neurotrophic factor (BDNF). PDGF is a potent mitogen for cells of mesenchymal origin and plays a significant role in angiogenesis, a process which has recently been recognized as crucial in growth and survival of neoplastic cells of the hematopoietic system. BDNF acts on certain neurons of the central nervous system and the peripheral nervous system and has a wide role in neuroprotection and neuroregeneration. However, the exact roles of PDGF, BDNF and their receptors in normal and malignant hematopoiesis remain unclear.

Aims: In this study, we aimed to investigate the levels of PDGF-AA and BDNF in plasma from CML patients and, where possible, to identify how TKI treatment affects these proteins levels.

Methods: Peripheral blood samples were obtained from newly diagnosed CML patients (n=5), CML patients treated with TKIs (n=5) and healthy controls (n=10). Informed consent was obtained from all subjects included in the study. Plasma PDGF-AA and BDNF levels were analyzed using LumineX technology with Human Neurodegenerative disease Panel 3 kit (Merck Millipore, Billerica, USA).

Results: We have observed that PDGF-AA levels were elevated in CML group (both before and during TKI treatment) compared to controls. Interestingly, we have noticed a decrease in level for newly diagnosed CML patients was higher compared to TKI-receivers (p <0.05). In case of BDNF, we have observed subtle changes between the tested groups: BDNF level in newly diagnosed CML subjects was lower compared to controls (p <0.05), but in TKI-receivers the level was comparable to control group (p >0.05). We have also tested one patient in different time points (at diagnosis, 3 months with TKIs, 6 months with TKIs) for both PDGF-AA and BDNF - we have observed PDGF levels drop and BDNF rise with time.

Summary/Conclusions: In our study we have demonstrated that PDGF-AA and BDNF are feasible targets for plasma proteomic analysis in CML patients, both for studying overall patterns of protein expression and also for identifying proteins differentially expressed before and during TKI treatment. We have shown that PDGF level drops down after TKI treatment, while on the opposite BDNF level in plasma raises with time in CML patients receiving TKIs. We have also monitored these proteins levels over time in the same patient (at the diagnosis and 2 months after start of treatment), but this group is too small for any meaningful conclusions. Further studies are required to elucidate the PDGF, BDNF and possibly other growth factors, neurotrophins and their receptors role in normal and malignant hematopoiesis.

PB1813

A CASE OF ATYPICAL CHRONIC MYELOID LEUKAEMIA WITH LATE DISCOVERY OF JAK2

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Background: Myeloproliferative neoplasms (MPN) include on the one hand chronic myeloid leukaemia defined by the presence of Philadelphia chromosome and BCR-ABL remodeling, and on the other hand 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 proposal of an entity 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+CM, or a BCR-ABL+CM during treatment for a V617F JAK2+MPN (3,4,5,6,7). A very small number of patients showed coexistence of those two mutations (8).

Aims: We report a 62y old woman patient with chronic myeloid leukaemia with late discovery of JAK2.

Methods: Clinical presentation: A 62-year-old man with no notable medical history was admitted in 2009 for CML. After failure of first line treatment by Imatinib in 2009 (poor tolerance and incomplete molecular response), treatment by Nilotinib was initiated in 2012 allowing for, to this day, good molecular response despite poor digestive tolerance in the form of dyspepsia. Ever since, the patient was treated with Imatinib for CML. In 2013, a JAK2 mutation was found positive on the basis of good molecular response and decreasing blood cell counts. The mutation was identified as V617F JAK2 which was followed then by polycythemia (Hb: 16.7–19 g/dl) that were first attributed to hemocentromisation and inflammation due to recurring bacterial urinary tract infections. Neither infiltration of the lymph nodes nor organomegaly had been noted. In 2014, the patient complained of abundant sweating in the absence of any signs of fever. A second MPN was suspected. V617F JAK2 mutation was found positive and treatment by Hydrea for essential thrombocythemia was initiated. Adaptation of Nilotinib posology was decided to avoid possible cytopenia due to its association with Hydrea.

Evolution: (see Figure 1A) As of the last follow-up consultation in 2017, BCR-ABL remains undetectable and the overall state of health was preserved. Hyperleukocytosis as well as myeloplasia were persistent on the blood count whereas hemoglobin and platelets had normalized. To determine whether or not V617F JAK2 mutation was present at the time of CML diagnosis, a 2009 sample, in which JAK2 V617F had been estimated at less than 1%, was reanalyzed by means of molecular biology in January 2017. This exam found the mutation in quantities below the clinical significance threshold (1%). But this positivity, however small (0.19%), shows preexistence of those two pathologies has not yet been genetically determined (8). Are those two independent pathologies or do they share a common tumoral clone? In this case, the JAK2 and BCR-ABL evolved in negative correlation and as such it is surmised that there were in fact two independent diseases, with two preexisting pathological clones at the time of the first diagnosis, treatment of the first pathology having been responsible for the proliferation of the second clone.
Chronic myeloid leukemia - Clinical

PB1814

E14A2 TRANSCRIPT IS ASSOCIATED WITH HIGHER PROBABILITY OF DURABLE TREATMENT FREE REMISSION IN CML PATIENTS

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Background: TKIs discontinuation in CML-CP patients with deep molecular response (DMR) are feasible, safe and 40-60% of them maintain treatment free remission (TFR); sokal risk score and duration of TKI-therapy were significantly associated with molecular relapse, according to Euro-Ski and STIM1 trials. While it is known that patients with e14a2 achieve earlier, deeper and more durable responses compared to those with e13a2, few information is available on the influence of the type of bcr-abl transcript on TFR duration.

Aims: Here we describe our single center experience of TKI discontinuation in CML-CP patients with sustained DMR.

Methods: Bcr-abl transcripts were determined by RQ-PCR analysis performed in accordance with EAC protocol (Gabert et al, Leukemia 2003) and to the standards of the Italian national network Labnet. All 174 CML-CP patients presently followed at our institution according to ELN guidelines and treated with 1st or 2nd TKIs were analysed: 103 (59%) had e14a2 and 69 (40%) e13a2 transcript (in 2 pz bcr-abl were not detectable). Criteria for TKI discontinuation was sustained DMR (MR4 or better) for at least 2 years. After TKI withdrawal, RQ-PCR for BCR-ABL was performed every month during the first year and every 2 months thereafter. TKI treatment was reintroduced immediately if DMR loss occurred. TFR was defined as the time between the date of TKI cessation and the date of restarting treatment for DMR loss or, if TKI was not resumed, the date of the last contact.

Results: Forty-nine patients, 25 male and 24 female, discontinued TKI treatment. At the time of discontinuation median age was 63 years (43-85), median time from TKI start 113 months (30-172), median duration of sustained DMR 60 months (24-153). Sokal distribution was 49%, 29% and 20% for low, intermediate and high risk (one patient was not evaluable). Among our 174 patients 39% (40/103) of all e14a2 patients and 13% (9/69) of all e13a2 discontinued TKI (P 0.0002, chi square). Thirty-six patients discontinued imatinib (11 of them with previous INF treatment), 13 stopped nilotinib (8 in first line, 5 in second line treatment). Median follow up after treatment discontinuation was 19 months (3-76), including 31 patients with follow up > 12 months. Thirty (26%) patients lost DMR. Median time off-therapy for these patients was 3 months (2-8), and only 1 lost DMR after 6 months. Therapy was restarted in all 13 patients (2 in MR1, 4 in MR2, 7 in MR3), 10 achieved a second DMR after a median interval of 2 months (1-7); 2/13 patients are in M3 after 7 and 12 months, 1 patient is still in stable DMR. 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 due to 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 a favorable molecular responder to IM had a trend for achieving a MR4.5 more frequently, efficacy than standard-dose IM for the treatment of patients who have suboptimal CRyR than group 3 (83.1% vs 72.8%, P=0.195) and group 2 (P=0.405).

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Background: Imatinib (IM) and its generic form are widely used as one of the standards of care for chronic phase (CP) chronic myeloid leukemia (CML). Although 7-year follow-up on the IRIS demonstrated the long-term prognostic values of molecular response at specific time points, achieving major molecular response (MMR) at 18 months showed minimal event-free survival (EFS) benefit, compared with not achieving MMR but having complete cytogenetic response (CCyR). In addition, the best treatment for these patients remains less clear.

Aims: In this study, we investigated the efficacy of nilotinib (NIL) versus high-dose IM versus sustaining standard-dose IM for the patients in CCyR with suboptimal molecular response to first-line IM therapy.

Methods: Early CP CML patients who have achieved CCyR but not MMR after 18 months of 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 400mg 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 enrollment was 36 months (range, 1-36), 45 months (range, 12-120), and 42 months (range, 12-120) 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 (no MMR after 12 months; n=14), in group 3, 22 patients switched to other treatment due to intolerance (n=7), lack of response (no MMR; n=12), failure (n=1), or switching to nilotinib (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 3 (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 MMR by 36 months showed a trend of higher in group 1 than the other two groups (11.7% vs 0% vs 2.6%, group 1 vs 2, P=0.066, group 1 vs 3, P=0.099, group 2 vs 3, P=0.405).

Summary/Conclusions: NIL 400mg twice daily treatment showed better efficacy than standard-dose IM for the treatment of patients who have suboptimal molecular response to first-line IM. Additionally, a switch to NIL in suboptimal molecular responder to IM had a trend for achieving a MMR more frequently, suggesting the potential benefit of a treatment-free remission.

PB1816

COMPARATIVE ANALYSIS OF PULMONARY HYPERTENSION IN THE 105 CML PATIENTS TREATED WITH IMATINIB, Nilotinib and Dasatinib

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Background: Pulmonary hypertension (PH) has been reported in 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 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 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 TRP (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 TRP>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 other 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-TERM 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 mutations. Detection of T315I mutations, one of the key TKI resistance mechanisms in CML pts, is prone to appear in a distinguished cohort of 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 24.95% 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 involved into clinical practice.

**Summary/Conclusions:** 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 analysis of resistance. We believe here that the regional difference of mutation profiles should also be considered. Therefore, to enable correct triggering of particular type 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 CT RESULTS IN A HIGHER MOLECULAR RESPONSE RATE AT 6 MONTHS COMPARED TO IM/ HYDROXYUREA. FINAL RESULTS OF THE CML2004 STUDY.**

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**Background:** IM is known to be an efficient and well-tolerated drug in chronic phase (CP). Hydroxyurea (HU) is effective in controlling elevated white blood cell counts and has been widely used to treat CML prior to the era of tyrosine kinase inhibitors (TKIs). The combinations of IM and HU have been tested in vitro and showed a additive suppression of CML CFU-GM cells. Combinations of IM with hydroxyurea cytostatic agent have been tested both in vitro and 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). Start- ing dose of IM was 500mg daily and was increased by 500mg to a maximum of 3,000 mg daily. According to protocol, 500mg IM 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 endpoint.

**Results:** In 2004 IM was approved by regulatory authorities in 2004. 110 patients with newly diagnosed CP-CML were included in the phase I study. Additional 93 patients were enrolled in the phase II of the study, 5 of whom were excluded. With ratio 2:1 in phase II, 88 patients were randomized to the IM/HU (n=59) and IM (n=29) arm, respectively.

**Summary/Conclusions:** Lower molecular response rates in patients with unknown transcript type that were observed at differences are 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 to the 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 available for 141 patients and was in high (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 assessed with the ELTS (2013) criteria was 41.1% (0.383) and at 12 months 41.9 (IM/HU) vs 58.9% (not significant). Time to event analyses of OS and PFS did not result in significant differences; neither did group comparisons between the probabilities of CCR and MMR. The median HU dose was 500mg (range 152-3000); the median IM dose was 400 mg (range 145-617mg). The gross number of adverse events in general or of adverse events of grade 4 were not different between the two arms, but cumulative incidences showed an earlier occurrence in the IM/HU than in the IM arm (p=0.0343, Gray test)

**Summary/Conclusions:** Compared to imatinib only, the combination of imatinib and HU resulted in a lower MMR rate at 6 months but a similar MMR rate at 18 months. Furthermore, IM/HU was associated with more early adverse events. There was no indication of a beneficial effect in the treatment of CML patients in 1st chronic phase using the combination of IM with HU.

**PB1820**

**A MULTICENTER, OBSERVATIONAL, AMBISPECTIVE STUDY EVALUATING EFFICACY AND SAFETY OF GENERIC IMATINIB COMPARED TO GLEEVEC IN CHRONIC MYELOGENIC LEUKEMIA IN CHRONIC PHASE - 3 MONTHS RESPONSE ANALYSIS**

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**Background:** The efficacy of branded imatinib (Gleevec) in the first-line treatment of chronic myeloid leukemia (CML) has been demonstrated in several clinical studies. However, there are few consistent data in the literature on the efficacy and adverse effects of generic formulations of imatinib. In Brazil, CML patients have been treated in the national public health system with generic imatinib since June 2013.

**Aims:** The present study aims to evaluate the efficacy and safety of generic imatinib in the treatment of CML in comparison with the reference drug (Gleevec) in the first three months of imatinib treatment.

**Methods:** This is a multicenter, observational, ambispective, cohort-type study. The study was initiated in January 2015 with the intended participation of 17 Brazilian centers. In the prospective group, were selected chronic phase CML Brazilian patients who started their first-line treatment with generic imatinib between January 2015 and October 2016, whereas retrospective group was treated with Gleevec between January 2010 and December 2011. All patients started their first-line imatinib less than six months from diagnosis. Data study were collected and managed using REDCap electronic data capture tools. Demographic data were collected at diagnosis: age, gender, Sokal, Hasford, EUTOS score, comorbidity, cytogenetics, BCR-ABL transcript type. The definition of the response followed the recommendations of the Chronic Myeloid Leukemia Net 2013 criteria. Adverse events were assessed based on the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.3, 2010. Statistical analysis: SPSS version 21.0 was used applying the chi-square and t-test, when adequate. All analysis considered p-value <0.05 as significant.

**Results:** Ten centers were included. One center registered 177 patients in the retrospective group and 68 patients in the prospective group so far. For this preliminary analysis, response data from 132 patients were available (47 from prospective and 85 from the retrospective groups). The median age of patients was 54 years in the prospective group and 46 years in the retrospective group (P=0.12). Sokal scores were 42% in the prospective group and 45% in the retrospective group (P=0.48). There was no difference between the groups concerning gender, Hasford, EUTOS scores, ECOG, blood cell counts at diagnosis and before starting treatment.

**PB1821**

**COMPLEX ADDITIONAL CHROMOSOME ABERRATIONS IN PH-POSITIVE CELLS IMPACT ON CHRONIC MYELOID LEUKEMIA PATIENTS’ SURVIVAL IN THE ERA OF TYROSINE KINASE INHIBITORS**

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**Background:** Additional chromosomal aberrations (ACA) as marker of clonal evolution in chronic myeloid leukemia (CML) patients were previously noted in association with resistance to therapy. The presents of ACA have been associated with a worse prognosis for survival in the pre-TKI era. The ACA classification proposed earlier was based only on its frequencies. Whereas ACA’s clinical impact had not yet been clearly established.

**Aims:** The aim of our study was to evaluate the long-term impact of the ACA presence in Ph-positive cells in CML patients on TKI treatment results.

**Methods:** 30 patients with ACA in Ph-positive cells treated in our center from 2005 to 2015 years were included in this study. Cytogenetic analyses of at least 20 Giemsa- and giemsa- bone marrow metaphases were interpreted per ISCN 2013. We analyzed overall survival (OS) and cumulative incidence of CML-related death on TKI treatment. Cox regression was used for multivariate survival analysis, that included next covariates: number of ACA, type of ACA, age, TKI type, CP or AP at diagnosis. OS was estimated by Kaplan-Meier method with log-rank test for comparison. Cumulative incidence of CML-related death was estimated into consideration the presence of competing risks (CML-unrelated death) using Gray’s test for comparison between groups.

**Results:** Median follow-up period in ACA group (n=30) was 51 months (3-124). ACA at diagnosis were detected in 16 (53%) of 30 patients. Chronic phase patients at diagnosis were determined in 23 (77%) patients. Imatinib was used as first-line in 20 (67%) patients, 3 (10%) patients were initially treated with Nilotinib. Accelerated phase was defined in 7 (23%) patients. In that group treatment of 6 patients was started with Imatinib and Dasatinib was given initially for one patient. «Major-order» ACAs (trisomy 8, +der(22)(q11), del(13)(q14), del(5q), del(9q)+der(9))(p22)) were detected in 16 (53%) of 30 patients. Complex aberrations (2 ACA and more) were revealed in 7 (23%) patients, 4 patients from this group had «major-order» 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.
The second group consisted of 48 patients switched from OI to GI, in which the rate of achieved therapeutic response at the time of switching and the rate of maintenance of CCyR, MMR and MR4 after a minimum of 12 months under therapy with GI were also 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 included 14/53 (26.6%) patients, while at 12 months ELN criteria satisfied 13/49 (26.5%) of analysed patients. After 18 months of therapy with GI the rate of CCyR was 35/46 (76.1%) and MMR was 28/45 (62.1%) and showed trend of increase. During the median follow-up period of 23.8 months 3 patients have progressed to blast phase and total of 7 patients died. In the second group, in time of switching from OI to GI, the rates of achieved CCyR, MMR and MR4 were 82.5%, 65.8% and 49% of patients respectively. The rate of maintenance of previously achieved CCyR was 95%, of MMR 88% and of MR4 72% in the course of the median duration of GI exposure of 37.8 months. When comparing first and second group respectively, the rates of patients which have been switched to 2nd generation TKI because of the failure or intolerance to imatinib were 27.8% vs 24.8%, and 60.5% vs 64.5% of them achieved secondary optimal therapeutic response (CCyR plus MMR), while 25% vs 20% of them have been sent to BMT. Group switched from OI to GI had not significantly different molecular AE compared to the GI group in both group (21.7% 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 cohort. Relationships between change of molecular response and quality of life scores 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 (waring or failure), and overall survival rate at 5 years was significantly higher in those who achieved optimal response at 12 months (P =0.047). Achieving optimal response at 12 months was associated with better role limit-ations (HR=0.39, 95% CI, 0.2-0.79; P =0.021) in CML-CP patients aged <40 years, female gender, and higher education level were also associated with better HRQoL subscales.

Summary/Conclusions: Achieving optimal response at 12 months was associated not only with longer overall survival and less treatment failure and disease progression, but also better HRQoL in newly diagnosed patients with CML-CP on front-line tyrosine kinase inhibitor.

PB1825

MULTI-COUNTRY RETROSPECTIVE CHART AUDIT STUDY TO EXAMINE DEEP MOLECULAR RESPONSE (MR4.5) ASSOCIATED WITH...
SECOND-LINE TYROSINE KINASE INHIBITORS IN CHRONIC PHASE - CHRONIC MYELOGENOUS LEUKEMIA (CML-CP)
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Background: Achieving deep molecular response, ≥4.5-log reduction (MR4.5; BCR-ABL1 on the International Scale [IS] ≤0.0032%), is one of the important prerequisites for attempting treatment-free remission. Limited information is available on comparative rates of MR4.5 between nilotinib and dasatinib in second-line (2L).

Aims: This study aims to investigate time to achieving MR4.5 and major molecular response (MMR; ≥3-log reduction or ≤0.1% in BCR-ABL1 on IS) in CML-CP patients (pts) treated with nilotinib vs dasatinib in 2L.

Methods: We performed a retrospective chart audit study with 2 panels composed of 11 experts recruited on oncologists (N=141) globally to conduct a retrospective medical chart audit. Physicians were instructed to select up to 4 pts who met the following criteria via a random letter generation scheme for the first letter of pt’s last name: diagnosed with CML-CP at age ≥18 years, initiated 2L nilotinib or dasatinib between 1/1/11 and 12/1/13, and had ≥12 mos of follow-up data after initiating 1L TKI. Multivariate Cox proportional hazards models accounting for country clustering random effects were used to assess the effect of nilotinib vs dasatinib on time to MR4.5 and MMR, adjusting for age, gender, Sokal risk score at diagnosis, hydroxyurea use before 1L TKI, ≥1 vs 2nd generation TKI as 1L, and reasons for 1L TKI discontinuation. Adjusted hazard ratios (HR) and 95% confidence intervals (CI) were reported. Adverse events (AEs) were also described.

Results: The study included 236 pts from Australia, Brazil, France, Germany, Italy, and Netherlands treated with nilotinib (N=115[49%]) or dasatinib (N=121[51%]) in 2L. Both groups had a similar mean follow-up of 23 mos, median age of 56 yrs, and 35% females. 1L nilotinib pts (N=59) and 1L dasatinib pts (N=72) were treated with the other 2nd generation TKI in 1L (p=0.01). A higher proportion of nilotinib pts had high-risk Sokal score (20.9% vs 11.6%, p=0.05) and received prior hydroxyurea (8.7% vs 3.3%, p=0.08) vs dasatinib. 85% and 11% of 2L nilotinib pts discontinued 1L TKI due to resistance and intolerance, respectively, prior to switching to nilotinib, vs 74% and 22% for 2L dasatinib pts (both p<0.05). The univariate Cox model showed that nilotinib had a non-significantly higher rate of achieving MR4.5 than dasatinib (32% vs 31% at 24 mos for 2L nilotinib and 2L dasatinib, respectively, based on the Kaplan Meier estimator; unadjusted HR=1.09, 95% CI [0.87, 1.38], p=0.46); however, after multivariate adjustment, nilotinib reached a significantly higher rate of achieving MR4.5 (adjusted HR=1.36, 95% CI [1.07, 1.73], p=0.01) than dasatinib. Among those who achieved MR4.5, 45% of nilotinib pts maintained MR4.5 for ≥1 yeal vs 39% of dasatinib pts (p=0.60). Additionally, higher-risk Sokal score (HR=0.31, 95% CI [0.14, 0.72], p=0.01) and resistance to 1L TKI (HR=0.60, 95% CI [0.24, 0.88], p=0.01) were inversely associated with achieving MR4.5. There was no significant difference in MMR achievement between 2L TKI groups. Over 3 times more dasatinib pts experienced pleural and pericardial effusion AEs than nilotinib pts (9.9% vs 2.6%, p=0.02). One nilotinib pt had ischemic heart disease-related AE vs none for the dasatinib group (p=0.49).

Summary/Conclusions: This retrospective chart audit study suggests that 2L nilotinib may be associated with a higher rate of MR4.5 than 2L dasatinib in CML-CP. Our results should be taken with caution as this study is susceptible to unmeasured confounding and biases due to its retrospective and observational nature. Rigorous clinical assessment in a prospective setting is needed to conclusively rates of patients achieving MR4.5.

PB1826
COMPUTATIONALLY INTELLIGENT PREDICTION OF CLINICAL OUTCOME IN CHRONIC MYELOGENOUS LEUKEMIA
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Background: Computational intelligence has been applied to a wide range of problems, to assist in decision-making, especially artificial neural networks, fuzzy systems and powerful hybrid neuro-fuzzy approaches have already proven their strong potentials in medicine. Despite that, applications in hematology are still scarce.

Aims: In this study we have developed novel ANFIS neuro-fuzzy prognostic models based on clinical and morphometric diagnostic data, to enable better prediction of complete cytogenetic response (CCgR) for patients with chronic myeloid leukemia.

Methods: This prospective study included a consecutive series of patients with chronic myeloid leukemia (CML) who were started on imatinib therapy. Analysis was performed on 32 patients (age: 10, 12, and 18 months as the outcome variables). A total of 40 patients on imatinib therapy were included in the final analysis. Of these, 25 (62.5%), 29 (72.5%), and 32 (80%), respectively, achieved CCgR at 6, 12, and 18 months after initiation of imatinib. Computationally intelligent neuro-fuzzy models that were developed included EUTOS score on diagnosis and one of the following morphometric parameters: microvascular density, length of the minor axis, area or circularity of the blood vessel. Adaptive neuro-fuzzy systems represent a specific combination of artificial neural networks and fuzzy logic, thus combining the learning ability of artificial neural networks with the knowledge representation capability of fuzzy logic systems. ANFIS (Adaptive Neuro Fuzzy Inference System) consists of five layers of nodes (neurons), whose 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 form 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 ANFIS model, including EUTOS score and minor axis morphometric parameter was better than a model that includes only EUTOS score and regression model based on the same inputs. Overall model correct classification achieved for EUTOS, two input LR model and two 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 MYELOGENOUS LEUKEMIA OUTSIDE CLINICAL TRIALS
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Background: In December 2014 the oral tyrosine kinase inhibitor (TKI), ponatinib was granted an accelerated approval by the FDA based on promising results from the phase II PACE (Ponatinib Ph-ALL and CML evaluation) trial. Yet, nowadays the use of this drug is limited because of safety issues, most frequently cardiovascular (CV) events. In this study we aimed to assess the safety profile and efficacy of ponatinib outside clinical trials.

Methods: Data from electronic charts of chronic myeloid leukemia (CML) patients treated with ponatinib were analyzed.

Results: Patients characteristics: Between 4.2011 and 1.2017 (69 months) 37 patients with an initial diagnosis of CML in 9 medical centers in Israel received ponatinib. The median age at time of treatment was 43 years (range: 9 to 82) and approximately half of the patients had chronic phase CML (N= 19, 53%). Based on their medical history, 36% (N=12) were at increased risk for vascular complications. Pre-ponatinib treatments: Patients received at least one other TKI and most received at least-two different TKI.
based regimens (N=28, 76%). Nine patients (25%) underwent hematopoietic stem cell transplantation (HSCT) prior to ponatinib. The time that lapsed from diagnosis until ponatinib initiation ranged considerably (from 1 to 215 months, median 47 months). *Indications for ponatinib switch*: 26% of patients (N=9) switched to ponatinib because T315I mutation was detected. The remaining switched either because of progressive disease, i.e. accelerated (N=5, 14%) or blastic (N=4, 17%) phases, and 14 (39%) because they experienced loss of previous molecular or cytogenetics response. Only 5% (N=2) switched because of unacceptable side effects to previous treatments. *Treatment with Ponatinib*: Patients received ponatinib for a median time of 14 months (range: 1 to 51). The drug started at the recommended dose of 45 mg/day only in 60% (N=22) of patients and in 24% of them (N=9) the dose was reduced during treatment. The median survival time of patients with ponatinib was 38 months (95%CI: 30 to 47 months) (Figure 1). Patients died because of cerebrovascular event (N=1), sepsis (N=2) or graft vs host disease that developed shortly after HSCT. *Response assessment*: Response assessment was available for 32 patients (86%). Seventy percent (N=22) achieved molecular response, of which 60% (N=13) achieved at least major molecular response. The median time to maximal response was 7 months (range: 3 to 28 months). *Drug discontinuation*: Twenty four percent (N=9) discontinued ponatinib after a median of 7 months (range: 1 to 18 months) because of disease progression (N=6) or severe adverse effects in two patients (cerebrovascular event and severe pancytopenia).

**Figure 1.**

**Summary/Conclusions:** In our cohort ponatinib was almost always used in patients who experienced treatment failure to previous TKIs. Still, molecular response was achieved in most patients, even in those with progressive disease in accelerated or blastic phases. The vast majority of patients received reduced doses of ponatinib and although more than one third of patients were at-risk for vascular events, only two patients developed serious life-threatening vascular episodes. In heavily pre-treated patients, ponatinib is effective and safe and can be considered even in patients with cardiovascular risk factors.

**PB1828**

**MOLECULAR RESPONSE TO THERAPY WITH TYROSINE KINASE INHIBITORS IN PATIENTS WITH BCR-ABL1(+ CHRONIC MYELOID LEUKEMIA PRESENTING WITH AN ISOLATED THROMBOCYTOSIS AT THE ONSET**

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**Background:** Generally, chronic myeloid leukemia (CML) and essential thrombocythemia (ET) are characterized by distinctive clinical and laboratory charateristics, including the spectrum of genetic abnormalities - Philadelphia chromosome (Ph) and BCR-ABL fusion transcripts in CML and JAK2, CALR or MPL gene mutations in ET. Therefore, even in the presence of overlapping features in some cases, the correct diagnosis can be assigned. However, in rare cases Ph chromosome and BCR-ABL fusion transcripts can be found in otherwise typical ET patients. The number of reported cases related to the subsequent course of the disease and the response to tyrosine kinase inhibitors (TKI) in such patients with BCR-ABL1-positive thrombocythosis is largely unknown.

**Aims:** To report the clinical course and response to TKI in patients (pts) with CML presenting with isolated thrombocythosis at the onset.

**Methods:** In total, 31 pts with Ph(+) and/or BCR-ABL(+), isolated thrombocythosis and a moderate or absent leukocytoysis were retrieved from the hospital database. The cohort comprised 17 females and 14 males, at a median age of 47 years (range 23-86). Diagnosis was based on blood and bone marrow morphology and differential, cyto genetics and/or molecular testing according to the WHO criteria (2008). Molecular monitoring was carried out using Xpert BCR-ABL Monitor or Xpert BCR-ABL Ultra tests (Cepheid). In total, follow up data for at least 6 months (mean 65 months) are available for 25 patients treated with TKI as a first-line therapy.

**Results:** At diagnosis the median leukocyte count was 22 x10^9/l (range 6-36) and platelet count – 1316x10^9/l (range 770-2815). Spleenomegaly was found in 5 pts (16.1%). Only one patient was diagnosed in accelerated phase as the remaining presented in chronic phase at diagnosis. Interestingly, 4 pts (12.9%) had a history of an antecedent solid tumor. All patients enrolled in the study were BCR-ABL1(+), b2a2 (n=16) or b2a2 (n=15). Karyotypes were available in 15. 10 of them had classical Ph in 16 of them (69%), with mutations (21.7%) to a cryptic translocation was detected as well as a variant Ph in the remaining 2 pts (8.7%). Imatinib was used as a first line therapy in 15 pts and optimal response was achieved in 53.3% (n=8), while 5 were switched to a second line, and 2 - to a third line therapy. First-line treatment with nilotinib in 11 pts resulted in optimal response in 80% (n=8). In patients with optimal molecular response (MR) was achieved in 80% (n=20), including deep MR in 56% (n=14). One pt was lost of follow up after optimal response was registered. No response was documented in 4 pts (16%) and progression to blast crisis developed in 2 of them. The mean OS was estimated 143 months and the cumulative proportion surviving at 5 years was 91%.

**Summary/Conclusions:** Interestingly, CML presenting with isolated thrombocytopysis at diagnosis in our cohort had high proportion of antecedent malignancies and high incidence of cryptic Ph translocation without any specific correlation with the transcript types. However, the clinical course and molecular response to TKI therapy was similar to the reported in CML in general. Acknowledgements: Partial support by the National Science Fund.

**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 breakdown products of the Philadelphia chromosome) is important in CML monitoring tumor burden. The International Scale (IS) was established to standardize reporting relative to a common baseline. As newer TKI therapies create deeper responses, analytical sensitivity has become a critical topic in investigations into TKI discontinuation, where researchers require a clinically validated assay that can distinguish a molecular reduction (MR) of ≥4.5 logs below baseline (i.e. MR4.5 or 0.0032%IS).

To clinically validate the Quantidex qPCR BCR-ABL1 IS kit and to reaffirm the clinical utility of BCR-ABL1 RT-qPCR monitoring in patients with (0;22) positive CML, a correlation between molecular response (MR) values and long-term outcome was determined.

**Methods:** The Quantidex qPCR BCR-ABL IS kit uses standard TaqMan chemistry to quantify BCR-ABL1 and the ABL1 reference gene. Associated software reports an international scale BCR-ABL1 value and a log-transformed MR value, with a 3 log-reduction from pre-treatment baseline representing a 0.1%IS or MR3.0. Three laboratories performed BCR-ABL1 testing on banked RNA specimens from 96 chronic phase CML patients from 2 hospitals drawn 12-18 months after starting TKI therapy. Clinical events (TKI therapy change, loss of complete hematologic or cytogenetic response, progression to accelerated phase or blast crisis, kinase domain mutation, or death) were recorded through 36x4 months after starting TKI therapy. 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.

Of 51 patients had MR>3.0 at 12-18 months post-TKI. Of these 51 patients who did not achieve a major molecular response (MMR), 20 had a subsequent clinical event, 17 had no event, and 14 were lost to follow-up (LTF). 45 patients had MR>3 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 LTF. 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 MR3]. 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 treatment. The Quantidex qPCR BCR-ABL IS Kit has excellent reproducibility and analytical sensitivity, and the achievement of MR3 (major molecular response) by this assay predicts prolonged event-free survival in TKI-treated CML patients.
PB1830

SHOULD SWITCHING TO SECOND GENERATION TKIS BE A RULE IN PATIENTS WITH CP-CML AFTER 3-6 MONTHS OF IMATINIB TREATMENT?: RETROSPECTIVE ANALYSIS OF CML PATIENTS TREATED IN A SINGLE BRAZILIAN CANCER CENTER

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Background: Early molecular response is an important predictor for survival and therapy-free remission in chronic myeloid leukemia (CML). The current guidelines define BCR-ABL1 >10% at 3 months and/or 1-10% at 6 months as warning signs; however, it is not clear if switching imatinib to second generation TKIs in this scenario improves responses and overall survival in patients outside clinical trials.

Aims: To analyze the proportion of patients with major molecular response (MMR) at 12 months according to the molecular response at 3 and 6 months in a cohort of CML population, not enrolled in clinical trials and treated only with imatinib. Also evaluate the incidence of molecular responses log3.0, log4.0 and log4.5 at any time in patients who did not switch to second generation TKIs.

Methods: Retrospective analysis of all 226 patients diagnosed with CML from January 2007 until January 2015 in our hospital. The exclusions criterias 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 8%, 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 <1% at 3 months and/or <1% had a higher probability of achieving MMR3, MMR4 and MMR4.5 at any time.

Figure 1.

Summary/Conclusions: Our study shows that around 30% of the patients that do not fail to imatinib at the first year of treatment may be late responders. Not all patients should change therapy, if they have not reached MMR at 12 months. Molecular response at 3 or 6 months might guide the decision to switch TKI, but patient’s comorbidities, possibility of discontinuation and cost of therapy should also be considered.

PB1831

PREDICTIVE PARAMETERS FOR IMATINIB FAILURE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Background: Development of tyrosine kinase inhibitors (TKIs) has significantly changed natural course of chronic myeloid leukemia (CML) and increased 10 year overall survival from 10-20% to 80-90%. Until recently, imatinib was the standard first-line treatment in CML. In 2013, nilotinib and dasatinib were approved as alternative front-line options. However, none of three TKI has been shown to have a clear survival advantage so this raised a debate on treatment selection. The early identification of patients expecting poor outcome is crucial for offering an alternative TKI regimen.

Aims: to analyze predictive parameters for Imatinib response as first-line treatment of CML patients.

Methods: The study was conducted on 168 consecutive patients with chronic phase of Ph+ CML who were diagnosed and treated at single university hospital from December 2000-January 2015. Following data were analyzed in terms of treatment response to Imatinib: demographic characteristics; currently used prognostic scores (Sokal, Hasford, EUTOS); liver and spleen size; laboratory parameters; influence of comorbidities analyzed by three scores (ACE 27, HCI, SCIRS); occurrence of second malignancies; conventional cytogenetic parameters; influence of comorbidities; 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%) achieved CCyR (83.3%); minorCyR, 16 patients (9.5%) had no cytogenetic response, 2 patients (1.2%) had hepatic toxicity verified by liver biopsy in the first six months of Imatinib treatment and 1 patient (0.6%) was lost from follow-up. After achievement of CCyR, 25 patients (19%) had a progression of disease by losing CCyR or development 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, leukocyte and platelet count, splenomegaly, eosinophils and basophils in peripheral blood were not found to be statistically significant for the Imatinib failure. Cox regression analysis identified hepatomegaly (p=0.001), leukocytosis>100x10⁹/L (HR=3.158; 1 point), blasts in peripheral blood >1% (HR=2.912; 1 point), and presence of additional cytogenetic aberrations (ACAs) (p=0.002) as a predictors of Imatinib failure. Accordingly, we assigned risk scores based on hazard ratios (HR) to hepatomegaly (HR=4.089; 2 points); leukocytosis >100x10⁹/L (HR=3.158; 1 point), blasts in peripheral blood >1% (HR=2.912; 1 point), and presence of ACAs (HR=11.110; 2 points). A final 3-tiered prognostic model named IMA-FAIL was thus developed, as low (score 0), intermediate (score 1-3), and high risk (score 4), according to which imatinib failure had 17% (8/47) of patients in low, 34.9% (30/86) in intermediate and 76.7% (23/30) in high risk group (HR=11.973, 95% CI for HR 2.237-7.053, p<0.001). In addition, presence of comorbidities as well occurrence of second malignancy were not predictors for Imatinib failure.

Summary/Conclusions: Hematologists are facing with challenge of making decision which TKI to choose upfront with increasing a chance to achieve best possible response. The new score allows better selection of patients who are suitable for treatment with Imatinib and may guide the clinical decision for front-line treatment of CML.

PB1832

A MULTICENTRE AUDIT OF SYMPTOMS AND QUALITY OF LIFE IN IRISH CML PATIENTS ON TYROSINE KINASE INHIBITORS

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Background: The development of tyrosine kinase inhibitors (TKIs) over the last 20 years has dramatically improved the outcomes for patients with every stage of chronic myeloid leukaemia (CML). Since the approval of the first TKI, imatinib, in 2001, there are now currently 5 oral TKIs available. Three are approved for frontline use (imatinib, dasatinib and nilotinib) and 2 others (bosutinib and ponatinib) approved for intolerance or failure of prior TKI. Current guidelines recommend patients to continue TKI treatment indefinitely; however, 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 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, which assesses interference of symptoms, as well as 6 interference items. The questionnaire took on average 5mins to complete and asked patients to rate their symptoms on a scale of 1-10 as experienced over the preceding 24 hours.
Results: Of the 87 patients surveyed, the most commonly prevailing symptoms were 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 TKIs frequently experience chronic adverse events with other TKIs. The 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 according to the score of Sokal was used. The main criterion of efficacy was the MMR. The considered costs were: the cost of the TPE program, established on the basis of the French health insurance reimbursement per patient and the costs associated with the use of “supplementary” care (examinations, consultations and additional hospitalizations). The point of view was from French health insurance.

Results: Over the 12-month follow-up period, the number of patients in MMR was similar between the 2 groups (9 in IG versus 8 in CG). However, the average time to obtain the MMR was significantly shorter in IG (6.9 months±3.8) than in CG (11.3 months±2.1) (p <0.05). The mean duration of MMR maintenance over the 12-month follow-up period was significantly longer in IG (3.2 months±3.5) than in CG (1.5 months±1.9) (p <0.05). Regarding the use of additional care, unexpected hospitalizations were significantly more numerous in CG than in IG (versus 0). Thus, costs associated with use of additional care were significantly lower in IG (€ 3,566) than in CG (€ 12,709). Thus, € 250 invested (annual allowance per patient) in the TPE saves € 508 in the use of care and reduces the time required to obtain a MMR by 4.4 months.

Summary/Conclusions: Thus, TPE is clinically and economically beneficial in our study population. By increasing the patient capacity to adapt to the treatment through the development of skills and adaptation processes, TPE reduces the costs of seeking care while improving the clinical response to treatment with a faster and more sustainable major molecular response.

PB1834
ROLE OF ALLO-HSCT IN THE TREATMENT OF PATIENTS WITH T315I MUTATION IN THE TKI ERA

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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.

Aims: To evaluate the results of different treatment modalities in CML patients with T315I mutation.

Methods: Retrospective analysis of 53 BCR-ABL T315I –positive CML patients (pts) was done.

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Aims: To evaluate the results of different treatment modalities in CML patients with T315I mutation.

Methods: Retrospective analysis of 53 BCR-ABL T315I –positive CML patients (pts) was done.
Methods: This cross-sectional study comprised 85 patients with CML in chronic phase, treated with imatinib, at KG Clinic for Hematology, Clinical Centre 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 TSH >40 mIU/L. Patients with a medical history of thyroid dysfunction were excluded. The duration of TKI treatment varied from 2 months to 10 years. The dose of imatinib was 400 mg daily, while nilotinib was dosed 800 mg a day.

Results: From the total number of patients included, 37 (43.53%) were female and 48 (56.47%) male. The median age was 54 years (range 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 mIU/L). Hypothyroidism was more common in males (71.5%, p=0.29, 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 sample. 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 INITIALLY TREATED WITH IMATINIB: 11 YEAR EXPERIENCE OF A TEACHING HOSPITAL

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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 the general population. Newer TKIs are associated with faster and deeper treatment responses, but have a more toxic side effect profile as well as being more costly.

Aims: This study looks at the 11 year experience of a single teaching hospital treating a population of almost one million and presents the response and survival data of this unselected population of patients with CML treated with imatinib as initial therapy.

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 2013 was selected to allow for adequate follow-up.

Results: In total 83 patients were newly diagnosed in this time period. Four patients, treated on SPIRIT2 with dasatinib as initial therapy, have been excluded from the subsequent analysis, leaving 79 patients treated initially with imatinib 400 mg daily. The median age at diagnosis was 53 years (range 13-93) with 40% female and 60% male patients. The median follow up was 6 years (range in living patients 29-163 months). Fifteen patients have died (19%). The median age at diagnosis of these was 73 years. Two deaths were transplant-related, both in patients who had failed available TKIs and had mismatched transplants. The only treated patient who died of accelerated disease was intolerant of all TKIs and unfit for transplant. Three patients died of other malignancies (ovarian, bowel and melanoma). Seven patients were transplanted. Of the surviving 5, 2 had sibling transplants early in the TKI era, 2 had MUD transplants after failing imatinib prior to the availability of second line drugs, and one failed to make an adequate response to imatinib then nilotinib and received a second line transplant. A selected population, up front imatinib with appropriate response (BCRABL:ABL ratio <0.1, 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, 6 have taken a pragmatic approach: three are treated 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 (BCRABL:ABL ratio <0.001, 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 a selected population, up front treatment 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.
Enzymopathies, membranopathies and other anemias

Enzymopathies

PB1839
CHARACTERIZATION OF HEMATOPOIETIC SAMPLES FROM PYRUVATE KINASE DEFICIENCY PATIENTS
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Background: Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. PKD produces chronic non-spherocytic hemolytic anemia, which can be fatal during early childhood and may result in lifelong transfusion dependence that in some instances persists despite therapeutic splenectomy. Although not considered a standard-of-care, allogeneic bone marrow transplantation has been curative in a limited number of cases. These findings, and the evidence that PKLR gene mutations are likely the sole etiology of the disorder, make PKD a candidate for gene therapy. Our lab has developed a therapeutic Orphan Drug lentiviral product (EMA: EU/3/14/1330; FDA: DRU-2016-5168) for the treatment of PKD and is working to develop an efficient and safe gene therapy clinical trial for the treatment of PKD.

Aims: In order to improve this new treatment, a more deep knowledge of the disease and its associated pathophysiology is necessary.

Methods: To characterize the hematopoietic profile of this disease, we have standardized flow cytometry protocols to perform both a qualitative and quantitative study of different population subsets. These included subsets of the hematopoietic stem cell compartment, erythroid progenitors, reticulocytes, mature erythrocytes and other mature lineages. Human routine samples consisted of peripheral blood, bone marrow and cord blood from PKD patients. In addition, xenogenic engraftment studies in immunodeficient (NSG) mice were also performed.

Results: Flow cytometry studies showed a clear imbalance in the erythroid populations. On the other hand, human PKD progenitors were able to engraft into NSG mice demonstrating that the disease does not likely impair hematopoietic stem cell capabilities.

Summary/Conclusions: Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disease caused by mutations in the PKLR gene. Our lab has recently developed a therapeutic Orphan Drug lentiviral product for the treatment of PKD. In order to improve this new treatment, we are also working to deep into the knowledge of the disease and its associated pathophysiology. Flow cytometry studies have shown a clear imbalance in the erythroid populations. Functionally, results in NSG mice we have demonstrated that the disease does not likely impair hematopoietic stem cell capabilities.

PB1840
OSMOTIC GRADIENT EKTACYTOMETRY: A VALUABLE SCREENING TEST FOR HEREDITARY SPHEROCYTOSIS AND OTHER RED BLOOD CELL MEMBRANE DISORDERS
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Background: Red blood cell (RBC) membrane disorders constitute one of the major causes of chronic hereditary hemolytic anemia. Main RBC membrane disorders, namely hereditary spherocytosis (HS), hereditary elliptocytosis (HE) and hereditary stomatocytosis (HST), alter membrane cohesion, membrane mechanical stability, and RBC volume, respectively. As a consequence, RBC deformability is compromised leading to their premature removal from circulation, manifested as hemolytic anemia. New generation osmotic gradient ektacytometry has become a powerful procedure for measuring red cell membrane deformability and therefore for the diagnosis of red blood cell membrane disorders.

Aims: The aim of this study is to evaluate osmotic gradient ektacytometry as an adequate assay to perform screening of membranopathies, focusing on the differential diagnosis between HS and non-spherocytic membrane defects such as HE and dHST.

Methods: A total of 75 patients with chronic hemolytic anemia oriented as hereditary RBC membrane disorders (hemoglobin disorders discarded and negative Coombs test) were included during a period comprised between January 2015 and August 2016. Normal controls were obtained from blood donors. Osmotic gradient ektacytometry was performed using the osmogam module of the Laser-assisted Optical Rotational Deformability Cell Analyzer: LoRRCa MaxSis (RR Mechatronics). Evaluation of osmogam parameters

adverse event (AEs), the 5-year event-free survival (EFS) and 5-year overall survival (OS) were all evaluated. Clinical features of the patients in different age groups are summarized in Table 1.

Results: The patient cohort consisted of 94 patients with median age of 53.4 years (range 18-78), with a slight predominance of females of 53.2%. There were more patients with intermediate and high Sokal scores in the EP group than in the groups MA and YA (p<0.001). To the contrary of that, most patients with high EUTOS score were observed in the group YA compared to MA and EP groups (p<0.001). The three groups were balanced regarding Euro score. The median duration of imatinib therapy was the longest in MA group (61.4 months vs 40.6 months in YA and 38.2 months in EP patients p<0.001). Furthermore, median follow-up duration was also the longest in MA group (64.3 months vs 48.5 months in YA and 44.7 months in EP patients p<0.001). The rates of complete cytogenetic response (CCyR) were similar in all three analysed groups (80.6% in YA, 86.5% in MA and 75.9% in EP, p=0.328) while rate of major molecular response was the highest in the MA group (83.3% vs 63.3% in YA and 57.1% in EL, p=0.001). The percentages of patients who switched to second-generation TKIs were similar in all three groups (36.7% in YA vs 30% in MA vs 32.1% in EP, p=0.559). There were the most of non-hematological AEs all grades in EP group (25% vs 13.3% in YA and 13.8% in MA, p=0.005). Hematological AEs also were common in EP group but not statistically significant (17.8% vs 10% in YA and in 12.1% in MA, p=0.156). The 5-years EFS in the MA group (88% (95%CI 82.1-96.9)) was significantly higher than in YA group (65.3% (95%CI 59.1-71.8)) and in EP group (60.2% (95%CI 49.5-73.7)). The 5-years OS in the EP group (74.7% (95%CI 65.9-89.0)) was significantly lower than in YA group (93.1% (95%CI 87.2-99.5)) and in MA group (90.8% (95%CI 85.8-97.8)). The number of deaths, both CML related or not, was the largest in the EP group (25% vs 13.3% in YA and 13.8% in MA, p<0.001).

Table 1. Clinical features of the patients in different age groups.

Summary/Conclusions: Results of this study indicate that age at diagnosis impacts the course of chronic myeloid leukemia treated with imatinib. The best clinical outcomes have middle age patients in terms of the highest rates achieved optimal therapeutic response and longer survival without events and overall survival. The degree of therapeutic responds in the elderly is comparable with that observed in younger patients, but the presence of comorbidity and more frequent occurrence of adverse events were affecting relatively lower overall survival. Although it might be expected that younger patient population has a better clinical outcome than patients middle age, a possible cause of poor outcomes is probably a late diagnosis at an advanced stage of the disease.
robustness for HS diagnosis was performed using the receiver operating characteristic (ROC) curve analysis. The optimal cut-off was determined as the one with the highest likelihood ratio. Statistical analysis was opred with GraphPad Prism.

Results: Specific patterns of osmolar LoRRCa MaxSis were observed for each individual membraneopathy. All HS curves were bell shaped but two different profiles were identified both presenting decreased Omin, and decreased Elmax and AUC. HE curves showed a characteristic trapezoidal shape with a decreased Elmax, Omax and AUC. dHSt curve was bell shaped with a specific decrease in Othyper 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 Elmax was the parameter that better separated HS from normal controls and dHSt, while the Omin was the best to separate HS from HE. The optimal Elmax cut-off to differentiate HS from HE was 159.0 (sensitivity 95.38%, specificity 85.71%). Expressing the results as % of variation in relation to the mean of our normal controls, the best combination of parameters for HS diagnosis would be Elmax < -3% and Omin > +5.2%. This combination sensitivity 98.46%, specificity 99.42%, while the optimal Omin cut-off to differentiate HS from HE was >15.90 (sensitivity 95.38%, specificity 85.71%).

Background: The primary lesion in HS is loss of membran surface area due to defects of the membran protein. Cryohemolysis test and osmotic fragility (OF) test are used for screening. However no test for HS is 100% reliable. The eosin-5-maleimide (EMA) binding test based on flow cytometry. Eighty percent of the fluorescent-labelled EMA binds to band 3 protein which is lost in HS due to protein 4.1, spectrin and ankyrin deficiency. Thus these parameters to the fluorescent EMA test detects all the different forms of HS.

Aims: In this study we aimed to evaluate the concordance of EMA binding test with other diagnostic parameters for HS.

Methods: The patients with HS were diagnosed according to clinical findings for hereditary anemia, spheromagely and spherocytes in peripheral blood. Hemoglobin, reticulocyte count, total/direct bilirubin, spherocytes in blood smear (BS), EMA binding test, OF test, and cryohemolysis test were obtained from patients and control groups. Correlation between EMA, OF and cryohemolysis tests were evaluated.

Results: Twenty-five male, 17 female HS patients aged between 1.0-19.0 years and 38 male, 47 female healthy controls were evaluated. There were no differences between both groups in terms of age and sex (Table 1). The median (range) values of hemoglobin (%), reticulocyte count (%), mean corpuscular volume (µm²), MCHC (%) and total bilirubin level were shown in Table 1. Besides MCH and MCHC, where there were greater differences between the two groups. The results are shown as mean and standard deviation (Table 1).

Table 1. Comparison of Clinical Laboratory Findings in Hereditary Spherocytosis groups and Healthy Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy Controls (n = 85)</th>
<th>HS Patients (n = 62)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>42 male, 43 female</td>
<td>25 male, 17 female</td>
<td></td>
</tr>
<tr>
<td>age (yrs)</td>
<td>19.1 (1.0-38.9)</td>
<td>17.0 (1.0-20.0)</td>
<td>0.146</td>
</tr>
<tr>
<td>hemoglobin (g/dl)</td>
<td>13.5 (12.8-14.9)</td>
<td>13.2 (12.9-14.4)</td>
<td>0.793</td>
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<tr>
<td>reticulocyte, (%)</td>
<td>6.1 (3.8-34.8)</td>
<td>3.1 (0.1-6.1)</td>
<td>0.000</td>
</tr>
<tr>
<td>MCV, (fl)</td>
<td>87.3 (83.2-90.9)</td>
<td>83.2 (80.4-86.4)</td>
<td>0.161</td>
</tr>
<tr>
<td>MCH, (pg)</td>
<td>32.6 (30.7-34.5)</td>
<td>32.0 (29.7-35.0)</td>
<td>0.160</td>
</tr>
<tr>
<td>MCHC, (%)</td>
<td>35.0 (31.5-38.0)</td>
<td>34.0 (30.5-36.0)</td>
<td>0.167</td>
</tr>
<tr>
<td>EMA binding test</td>
<td>38.0 (30.0-45.0)</td>
<td>20.0 (15.0-25.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>OF</td>
<td>86.3 (82.0-90.9)</td>
<td>38.9 (31.5-46.0)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

M/F: Male/Female, FC EMAflow cytometric eosin-5'-maleimide, OF: osmotic fragility, HS; hereditary spherocytosis, MCHC; mean corpuscular hemoglobin concentration, MCV; mean corpuscular volume, BS; blood smear

Summary/Conclusions: In this study EMA-FC was more sensitive and specifically than osmotic fragility. However specificity and PPV of cryohemolysis was higher than other test. Also we showed moderate concordance cryohemolysis and EMA test.

Although high sensitivity and specificity of EMA test there were need to use other tests together with family history of patient, physical examination, evaluation of blood smear and several tests for HS diagnosis.

PB1843

ADVANCES IN DIAGNOSIS OF HEREDITARY HEMOLYTIC ANEMIAS: THERMOGRAVIMETRY COUPLED WITH CHEMOMETRICS

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Background: The differential diagnosis of hereditary hemolytic anemia is generally carried out by applying different diagnostic protocols depending on the specific congenital erythrocyte defects. Thermogravimetric analysis (TGA) coupled with chemometrics has recently been proposed as a rapid and cost effective diagnostic tool for β-thalassemia screening. This model, consisting of Parahoric Superimposed Discriminant Analysis (PLS-DA), permitted the discrimination of thalassemia patients and healthy individuals, using thermogravimetric curves of blood samples [1].

In this study, the capability of thermogravimetry in conjunction with a mutl-
tivariate statistical analysis was investigated for the screening of hereditary hemolytic anemias due to different erythrocyte defects.

**Methods:** Whole blood samples collected in K2 EDTA were obtained, after informed consent, from patients suffering from congenital hemolytic anemias and were analyzed using the thermostable 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 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 the most useful 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. However, the severity and symptoms of the different GD phenotypes ranges from fatal perinatal to asymptomatic adult disease, and the heterogeneity of its presentation contributes to both misdiagnosis and delays in diagnosis by clinicians unfamiliar with the disease. The Gaucher Earlier Diagnosis Consensus (GED-C) Delphi initiative determined which signs and patient co-variables were regarded by experts in GD as most indicative of GD types 1 or 3 in the early stages.

**Aims:** From the findings of the GED-C expert consensus, to generate a simple web-based point-scoring system (PSS) suitable for use across clinical specialties, that provides guidance based on patients’ presenting signs as to whether GD diagnostic testing is appropriate.

**Methods:** An anonymous three-round Delphi process, conducted among a global panel of 22 expert physicians, established consensus on which signs and co-variables may be important in early GD type 1 and, separately, in early GD type 3. In round 1, free-text responses provided by the panel were categorized by an independent administrator. This categorization was checked and consolidated into summary factors by the non-voting co-chairs. In round 2, the factors were rated for importance by the panel using a 5-point Likert scale (1 = not important, 3 = important, 5 = extremely important). Any factors assigned an importance score of ≥3 by >75% of respondents were then rated for agreement in round 3, using a 5-point 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 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.

**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 2000 and March 2016, were analyzed retrospectively. Patients, who were diagnosed G6PD deficiency were scanned by using hospital patient information system. The patients’ ages, the cities they lived, complaints and the stories of them were questioned. Complete blood count, serum AST, LDH, total and direct bilirubin levels of all the cases in the study were recorded. G6PD levels were measured by quantitative spectrophotometric methods in biochemistry laboratory. The World Health Organization (WHO) is divided G6PD enzyme deficiency into five classes based on enzyme activity levels and clinical findings.

**Results:** The distribution of the cities where the cases were living, was given on the map in Figure 1. Patients’ average age, hemoglobin, and G6PD levels were 26.4±5.62, 14.6±1.51, and 0.86±0.38 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 after the examinations due to hemolysis after favism and prolonged neonatal jaundice respectively. 6 patients (4.3%) were diagnosed of G6PD deficiency by screening because of family history, but they didn’t have any hemolytic episodes before. After the patients evaluated with their clinical history and hemolysis findings; 6 patients (4.3%), who had chronic hemolysis, was considered compatible with Class I variant. 128 cases were considered as Class II variants.

**Summary/Conclusions:** G6PD enzyme deficiency in Turkey is seen most frequently in the Mediterranean region and the prevalence of G6PD deficiency in Central Anatolia and Aegean regions seems to be over the Turkey average (2%). Nearly half of the patients had hemolytic anemia due to favism. It is followed by hemolysis due to neonatal hyperbilirubinemia and drugs. 128 (91.4%) patients who had severe G6PD deficiency with intermittent hemolysis, were considered as Class II variants.
CHARACTERISTICS AND MANAGEMENT OF AUTOIMMUNE HEMOLYTIC ANEMIA: A SINGLE CENTER STUDY WITH 32 CASES

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Background: Autoimmune hemolytic anemia (AIHA) is characterized by red blood cell destruction mediated with autoantibodies against RBC antigens. Most common type is warm AIHA which can be either 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 6 patients also had thrombocytopenia (<50000) 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 danaspar. 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 three patients who were treated with standard dose of Rituximab; two achieved CR and one did not achieve any response. One of two rituximab-treated patients relapsed at 26 and 60 months and re-treated by rituximab; still following with CR for 16 months.

Summary/Conclusions: Although corticosteroids are the first choice of initial treatment of AIHA, most of the patients relapse at follow up. Steroid dependency and intolerance are also challenging. Splenectomy is still a considerable option for second-line therapy because of its high response rates and long remission durations. Rituximab is the other effective second-line therapy option with similar response rates to splenectomy. Until prospective studies will be performed, retrospective data would help the clinicians to choose best treatment algorithm for AIHA.
**Gene therapy, cellular immunotherapy and vaccination**

**PB1849**

**DEMONSTRATION OF FUNCTIONAL SIMILARITY OF PROPOSED BIOSIMILAR ABP 798 TO RITUXIMAB**

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**Background:** Proposed biosimilars undergo comprehensive structural and functional characterization before they can be studied in confirmatory clinical trials. ABP 798 is being developed as a biosimilar to rituximab. The originator is approved for treatment of non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, severe rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis.

**Aims:** ABP 798 was compared with rituximab sourced from the European Union (EU). Quality attributes assessed included binding properties (CD20, C1q, FcRn, and Fc receptors), antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and induction of apoptosis.

**Methods:** Binding of ABP 798 and rituximab to the CD20 antigen was characterized using a cell-based CD20 binding assay utilizing the human B-lymphoblastoid, WIL2-S, cell line. A direct binding ELISA was used to assess the binding of the Fc domain of ABP 798 to C1q. Binding of the Fc moiety of ABP 798 and rituximab to FcγR, FcγRⅡa, FcγRⅡb, and FcγRⅢa (158V) were evaluated in an AlphaLISA® competitive binding assays. Binding to FcRn was evaluated by an AlphaScreen® competitive binding assay. ADCC activity was evaluated in a functional cell-based assay, with CD20 expressing WIL2-S cells used as target cells and NK92-M1 cells, stably transfected with human CD16 (FcγRⅡa [158V]), used as effector cells. CDC activity was evaluated in a functional cell-based assay using a CD20 expressing human B-lymphoblastoid WIL2-S cell line and baby rabbit complement. Induction of apoptosis was assessed by measuring activation of caspase 3/7 in SU-DHL4 cells, a CD20-expressing human B cell lymphoma cell line.

**Results:** Relative binding (%) was comparable between ABP 798 and rituximab (Table 1).

**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.

**Table 1.**

<table>
<thead>
<tr>
<th><strong>Assay</strong></th>
<th><strong>ABP 798</strong></th>
<th><strong>Rituximab</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>85-100</td>
<td>85-100</td>
</tr>
<tr>
<td>IgM</td>
<td>89-102</td>
<td>89-102</td>
</tr>
<tr>
<td>IgA</td>
<td>81-100</td>
<td>81-100</td>
</tr>
<tr>
<td>FcγRI</td>
<td>96-102</td>
<td>96-102</td>
</tr>
<tr>
<td>FcγRIIa</td>
<td>98-97</td>
<td>98-97</td>
</tr>
<tr>
<td>FcγRIIb</td>
<td>94-98</td>
<td>94-98</td>
</tr>
<tr>
<td>FcγRIIIa (158V)</td>
<td>80-67</td>
<td>80-67</td>
</tr>
</tbody>
</table>

The dose response profiles and relative activity for ADCC and CDC were similar (mean ADCC relative activity: ABP 798, 88%; rituximab, 86%; mean CDC relative potency: ABP 798, 103%; rituximab, 104%). The dose response profile for induction of caspase 3/7 was comparable between ABP 798 and rituximab.

**Summary/Conclusions:** The results presented here suggest that ABP 798 is similar to rituximab sourced in the EU in terms of biological activity across the range of tested functions. These results provide a firm foundation for further clinical development of ABP 798.

**PB1850**

**DELAYED EFFECT OF G-CSF ON THE CYTOKINE SECRETION THROUGH G-CSF MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS IN CHILDREN WITH CEREBRAL PALSY**

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**Background:** Granulocyte colony-stimulating factor (G-CSF) has been widely used to mobilize peripheral blood stem cells. In addition, it also has been tried to reveal the regenerative potential in various neurodegenerative diseases.

**Aims:** We investigated the short-term and delayed effects of infused G-CSF for peripheral blood stem cell (PBSC) mobilization on the various cytokine secretions in children with cerebral palsy (CP).

**Methods:** G-CSF (10μg/kg/dose) was administered subcutaneously for 4 days to the children with CP. In first group, blood levels of G-CSF, interleukin (IL)-6, IL-10, insulin-like growth factor (IGF-1), vascular endothelial growth factor (VEGF), and brain derived neurotrophic factor (BDNF) as well as mobilized total nucleated cell (TNC)/CD34+ cell counts in peripheral blood were compared between just before G-CSF injection (D+0) and 1 day after 4 days of G-CSF injections (D+5). In second group, cytokine levels were measured between D+0 and 1 month after 4 days of G-CSF injection (D+30). Cytokine levels were measured by enzyme-linked immunosorbent assay.

**Results:** Baseline levels of G-CSF were significantly increased (p=0.000) and IGF-1 decreased (p=0.011) at D+5 after 4 days of G-CSF administration compared to control group. In contrast, other cytokine levels including IL-6, IL-10, VEGF, and BDNF did not show any significant changes between before and after G-CSF administration. CD34+ cell counts (p=0.000) as well as TNC counts (p=0.000) were significantly increased from D+0 to D+5 in children who received G-CSF compared to children received placebo. Regarding delayed effect of G-CSF administration, G-CSF levels were significantly increased from baseline to D+30 (p=0.000), along with the increase IL-10 (p=0.035) and VEGF levels (p=0.011) and the decrease of IGF-1 levels (p=0.014).

**Summary/Conclusions:** G-CSF which administered to mobilize PBSCs could induce the delayed effects on the levels of G-CSF itself as well as other cytokines which could affect on the neuroregenerative potential. Further studies would be warranted to reveal the mechanism and clinical significances of these delayed effect of G-CSF or mobilized PBSCs.
Hematopoiesis, stem cells and microenvironment

**PB1852**

**PD-1 IS HIGHLY EXPRESSED ON MEMORY T-CELLS RESIDING IN BONE MARROW BUT NOT IN PE-RIPHERAL BLOOD IN HEALTHY INDIVIDUALS**

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**Background:** Recently memory T lymphocytes were shown to be a highly heterogeneous cell compartment comprising different phenotypes, functional activities, gene expression profiles and survival capacities. Phenotypically due to the differentiation stage and functional activities memory CD8+ T cells can be divided into: central memory cells (Tcm), central memory-like cells (TcmLM), effector memory (Tem) and terminal effector (Tte). The survival rate of peripheral memory (Tm) and terminal effector (Tf) and reside in bone marrow (BM) as long-lived persistent T cells [Mahnke YD et al., 2013]. Programmed cell death protein 1 (PD-1) is well known as a negative immune regulator of T cells that has detrimental effects on anti-viral, anti-tumor immunity, mediates tissue tolerance to protect against immune-mediated tissue damage. Currently anti-PD1 immunotherapies are among the most effective anti-cancer immunotherapies available. PD1 pathway blockade is a key therapeutic mechanism [Bousisios VA et al., 2014]. Understanding the influence of PD-1 pathway on memory T cells homeostasis in BM might be critical for improving treatment of patients with cancers and hematological malignancies, but is still not well understood.

**Aims:** To evaluate PD-1 expression on distinct memory T cell subsets in BM and PB of healthy donors.

**Methods:** The first portion of BM and a sample of PB were obtained from healthy donors (n=10, m=6, f=4) with age 37.5 (22-53) years old. Numbers of white blood cells (WBC) in BM and PB samples were evaluated by Sysmex XE-2100 hematology analyzer. 1*10^6 of WBC (excluded nucleated red blood cell) from BM and PB were stained using “lyse-wash-stain” standard protocol. The CD8-APC-Cy7, CCR7-PE-Cy7, CD28-PE, CD45RO-FITC, PD1-APC antibodies were used for cell activation and staining 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 TcmLM subset. Tcm, TcmLM, Tm, Tle, Median of Td, CD8+ cells were 3.9%,1.01%, 22.7%,5.39%, 42.7%,17.86%, 21.9%,4.07% and 2.6%,0.41%, 6.6%,25.59%, 12.7%,15.25%, 8.9%,0.825% in BM and in PB respectively.

**Summary/Conclusions:** We found higher frequencies of PD-1 expressing memory BM T cells compared to PB. This might point to the important roles of PD-1 in regulation of memory T cells homeostasis in BM. In physiological conditions PD-1 is thought to neutralize self-reactive naïve T cells that in its turn lead to restraining T cells activation and blockade the development of autoimmunity in BM. On the other hand low expression of PD1 on T cells in healthy donors (n=10, m=6, f=4) with age 37.5 (22-53) years old...
Results: The mean values of CEPCs subpopulation CD34+CD45negdimVEGF
FR2+ estimated in ALL, ST and Controls were 0.00380 (SE=0.00072), 0.00613
(SE=0.00146) and 0.002953 (SE=0.0004) respectively. The mean percentage of CD34+CD45negdimCD133+VEGF+R2+ in ALL, ST and Controls was 0.00331
(SE=0.00072), 0.00499 (SE=0.00113) and 0.002663 (SE=0.00037). The correlation of CD34+CEPCs showed statistical significant difference of CD34+CD45negdimVEGF+R2+ between the ST % BCR control (Mean 0.003174, 95CI of diff 7.716e-005 to 0.002672). In ALL the levels of CD34+CD45negdimVEGF+R2+ the 1st year after treatment completion were 0.00458(SE=0.0026), during 1-3 years 0.0031(SE=0.00066) and >3 years 0.003243(SE=0.00081). The levels of CD34+CD45negdimCD133+VEGF+R2+ during the 1st year after chemotherapy was 0.0092 (SE=0.0037), 1-3 years 0.0027(SE=0.00063) and >3 years 0.0033(SE=0.00081). Statistical significance results were calculated for the levels of CD34+CD45negdimVEGF+R2+ in ST group between the groups <1 year and over years’ post treatment(Mean Diff 0.007747, 95 CI of diff 0.0002441 to 0.01525). The study of body weight in ALL and ST groups in relation with CEPCs showed no statistical significant difference, although a negative trend between obesity and CEPCs was found in the ALL group and a positive one in the ST group. The same trend also appeared in BP between ALL and ST regarding the CEPCs, with hypertensive patients in ALL group having higher levels of CEPCs than the ST hypertensive individuals.

Summary/Conclusions: The higher levels of CEPCs were estimated in ALL and ST just after treatment completion with a gradual decrease as time passes. The highest percentages of CEPCs were evaluated in ALL patients with normal weight and blood pressure in contrast with the solid tumor group. Further investigation is necessary to highlight the importance of these data.

PB1855
HEMATOLOGICAL PARAMETERS IN NATIVE HIGHLANDERS OF LADAKH AGED 4-19 YEARS
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Background: High altitude (HA) has always intrigued physiologists because of the remarkable ability of man to adapt to the hostile environment. Hematological changes associated with HA exposure is believed to be driven by hypobaric hypoxia of HA. Majority of the studies on HA physiology and hematological adaptation have focused on the hematological adaptation in lowlanders visiting HA or have compared the hematological profile of native highlanders from Andes and Tibet with those of the neighboring lowlanders. These studies have mostly been directed towards adult population with no or little reference to children and adolescent age groups. Moreover these studies have been done mostly on the highlanders of Andes and Tibet with no data on Indian highlanders.

Aims: We aimed at assessing hematological parameters in native highlanders in the age group of 4- 19 yrs and compare the same with Indian lowland population as well as native highlanders across the Himalaya.

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 pre-
cations, blood samples were drawn from the ante-cubital 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, 10-12y, 12-15y, and children more than 15y). Appropriate statistical analysis was done to compare the hematological parameters between the stratified age groups as well as between boys and girls.

Results: A total of 197 girls and 193 boys were included in the study. The mean age of the subjects was 128±80 (means±STD) months. The mean hematocrit value increased with age (38.68±2.51% in <5 yrs age group to mean age of the subjects was 128±80 (mean±2SD) months. The mean hemoglobin concentration we found that the mean corpuscular hemoglobin concentration

PB1856
AGE VARIATION OF B-CELL PRECURSORS IN BONE MARROW: NORMAL VALUES AS A REFERENCE FOR MDS IN BRAZIL
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tology, DASA, 6Laboratory of Hematology, Laboratórios Fleurio, São Paulo, 7Laboratory of Hematology, Children's Cancer Hospital, Barretos Cancer Center, Barretos, 8Pathology, University of Campinas, Campinas, Brazil

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. Sta-
tistical analysis: multiple regression to analyse the dependence of BCS from the variables analyzed.

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 -0.40. Age explained alone 49.6% of the variance of % BCPs/total cells, while “laboratory” explained 5.2% and panel used explained only 0.8%. Age explained only 24.9% of the variance of BCPs/CD34+ cells.

Table 1.

<table>
<thead>
<tr>
<th>Age group</th>
<th>% BCP/total cells</th>
<th>BCP/CD34+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 years</td>
<td>3.0% (1.5-5.1)</td>
<td>2.8% (0.35-3.8)</td>
</tr>
<tr>
<td>7-18 years</td>
<td>1.4% (0.25-3.2)</td>
<td>0.4% (0.02-1.8)</td>
</tr>
<tr>
<td>19-55 years</td>
<td>0.84 (0.77-7.96)</td>
<td>0.12% (0.02-0.8)</td>
</tr>
<tr>
<td>&gt;56 years</td>
<td>0.07% (0.02-2.8)</td>
<td>0.00% (0.00-0.00)</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.

PB1857
PERISTIN/BIGH3 RATIO AS A PROGNOSTIC MARKER OF IDIOPATHIC THROMBOCYTOPENIA AFTER ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION FOR THE PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA
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Summary/Conclusions: The hematological adaptation of Ladakhi kids is different as compared to other native highlanders. There is also a significant difference in the hematological response to hypobaric hypoxia with growing age and between boys and girls.

Figure 1.

Summary/Conclusions: The hematological adaptation of Ladakhi kids is different as compared to other native highlanders. There is also a significant difference in the hematological response to hypobaric hypoxia with growing age and between boys and girls.

Figure 1.
Background: Disrupted hematopoiesis is life-threatening complication of allelic hematopoietic cell transplantation (allo-HCT). The interactions of haematopoietic stem/progenitor cells (HSPCs) and bone marrow (BM) microenvironment, niche(s), control the homeostasis of BM. TGF-β induced gene 3 (BIG3), one of BM extracellular matrix (ECM) which is produced by niche cells maintain the homeostasis and regeneration of BM.

Aims: We analyzed the relationship between the idiopathic thrombocytopenia after allo-HCT and the BM expression of periostin as the only paralogue of BIG3.

Methods: We reviewed twenty patients who transplanted with matched sibling donor for acute myelogenous leukemia at Kyungpook National University Hospital from January 2010 to August 2015. BM biopsy specimens at the time of day 28, day 90, day 180, and 3 at day 365. There was no significant difference between idiopathic thrombocytopenia and the expression of BIG3 or Periostin (p=0.128). However, BM idiopathic thrombocytopenia manifested the low periostin/BIG3 ratio (p=0.007). Acute GVHD was observed in 12 patients (60%) and chronic GVHD developed in 13 patients (65%). The development of thrombocytopenia does not differ according to acute and chronic GVHD (p=0.847) (Figure 1).

Summary/Conclusions: The periostin/BIG3 might represent the status of BM niche during the homeostasis and regeneration of hematopoiesis. High periostin/BIG3 ratio could predict the recovery of the idiopathic thrombocytopenia.

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 emission 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 anemia. Images were captured with a confocal microscope of a HPM-100-40-Hybrid detector and excitation at 405 nm (diode laser,80 MHz). In order to create equivalent images of the cytological smears, pseudo-colors were attributed to different lifetime ranges. Images were compared with May-Grünwald-Giemsa (MGG) stained smears.

Results: FLIM created highly contrasted images, where different cell types could be easily recognized by their similarity with MGG images. Erythrocytes exhibited the shortest lifetimes (210.4±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 depanecocytes revealed significantly elevated values (314.2±66.7 ps and 312.5±67.0 ps respectively). Regarding erythropoiesis, the cytoplasm of erythroblasts showed significantly shorter lifetimes (623.5±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 ps versus myeloblasts: 1166.4±287.9 ps). The same differences could be found in megaloblastic anemia. There were no significant differences between the FLIM values of the different cell types between normal hemopoesis and megaloblastic anemia.

Summary/Conclusions: The FLIM technique is easily applicable on unstained routine smears and revealed images of good quality permitting cell identification. It allowed also to distinguish between erythroid and myeloid precursors cells and indicates the major physico-chemical changes during the process of falcification.

PB1860

TWO HEMATOLOGICAL MALIGNANCIES, SIMULTANEOUS OR CONSECUTIVE OCCURRENCE. EXPERIENCE OF A CENTER

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Background: Numerous reports of coexistence or consecutive occurrence of hematological malignancies are found in the literature.

Aims: : this study reports cases of patients with two hematological malignancies 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 demographic characteristics, diagnoses, treatment and overall survival are shown.
on Table 1. There are three deaths because of refractory diseases. Five patients needed treatment for the first disease and nine patients needed treatment for the second disease. Four patients had treatment for both diseases.

Table 1.


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.

Figure 1.
ADVANCED HODGKIN LYMPHOMA PATIENTS WITHOUT LARGE TUMOR MASS – A NEW PROGNOSTIC SCORE IDENTIFIES PATIENTS WITH FAVORABLE OUTCOME

B. Diermaier1,2, B. Andjelic1,*, D. Antic1, N. Moya1, C. Gruchet1, L. Perrichot1, C. Tomowiak1, Aretrospective study was performed on 149 patients classical HL, diagnosed in the period June 1997-December 2011. All the patients were in clinical stage III or IV and didn’t have any tumor lesion of 5 cm or more in its longest diameter. The standard of initial care was 6-8 cycles of ABVD followed by radiotherapy. Prognostic relevance of age more than 45 years, gender, CS IV, presence of B symptoms, IPS score, ESR≥50 mm/h, Hgb <10.5 g/dL, WBC≥15,000/mm³ and lymphopenia (lymphocytes <600/mm³ or <8% of WBC count) were examined.

Results: The median age of analysed patients was 37 (range 17-80). The median follow up was 98 months. For the whole group 5-year event free survival (EFS) was 63.1% and 5-year overall survival (OS) was 80.6%. In univariate analysis, worse OS was found in patients older than 45 years (5-year OS 66.7% vs 87.8%), patients with CS IV (5-year OS 70.2% vs 87.0%), B symptoms (5-year OS 77.6% vs 89.2%), IPS≥3 (5-year OS 71.8% 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≥3 (5-year EFS 63.8% 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 multivariante Cox regression model 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 classified at risk of survival based on prognostic factors were merged. Finally, we developed prognostic model for identifying patients at low (0 factors), intermediate (1 factor) and high risk (2-3 factors) for poor outcome (p=0.000). According to this model, in the examined group 34 (22.8%) patients had low, 64 (43.0%) intermediate and 51 (34.2%) high risk for poor outcome, with 5-years OS of 100%, 85.7% and 62.9% respectively.

Summary/Conclusions: According to the score which we developed, ABVD is very effective in the subgroup of advanced HL patients without large tumor mass and without identified risk factors.

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 toxicity. The identification of patients who require more or less aggressive initial approach remains the main goal for many investigators in the field of HL.

Aims: The aim of this study was to identify among patients with diagnosed advanced HL without large tumor mass the subgroup which should not be considered for more aggressive approach than ABVD.

Methods: A retrospective study was performed on 149 patients classical HL, diagnosed in the period June 1997-December 2011. All the patients were in clinical stage III or IV and didn’t have any tumor lesion of 5 cm or more in its longest diameter. The standard of initial care was 6-8 cycles of ABVD followed by radiotherapy. Prognostic relevance of age more than 45 years, gender, CS IV, presence of B symptoms, IPS score, ESR≥50 mm/h, Hgb <10.5 g/dL, WBC≥15,000/mm³ and lymphopenia (lymphocytes <600/mm³ or <8% of WBC count) were examined.

Results: The median age of analysed patients was 37 (range 17-80). The median follow up was 98 months. For the whole group 5-year event free survival (EFS) was 63.1% and 5-year overall survival (OS) was 80.6%. In univariate analysis, worse OS was found in patients older than 45 years (5-year OS 66.7% vs 87.8%), patients with CS IV (5-year OS 70.2% vs 87.0%), B symptoms (5-year OS 77.6% vs 89.2%), IPS≥3 (5-year OS 71.8% 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≥3 (5-year EFS 63.8% 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 multivariante Cox regression model 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 classified at risk of survival based on prognostic factors were merged. Finally, we developed prognostic model for identifying patients at low (0 factors), intermediate (1 factor) and high risk (2-3 factors) for poor outcome (p=0.000). According to this model, in the examined group 34 (22.8%) patients had low, 64 (43.0%) intermediate and 51 (34.2%) high risk for poor outcome, with 5-years OS of 100%, 85.7% and 62.9% respectively.

Summary/Conclusions: According to the score which we developed, ABVD is very effective in the subgroup of advanced HL patients without large tumor mass and without identified risk factors.
Methods: In study were included 85 previously untreated patients, presented with classical HL between 2002 and January 2016. This retrospective study did not require approval by the Local ethical committee. Inclusion criteria were: a histologically confirmed diagnosis of classical HL, the presence of a fixed in paraffin before treatment a lymph node sample or other diseased tissue, the minimum follow-up was not less than 18 months.

Results: In the study population (n=85) identified 30 (35%) histological samples bcl-2+, and 55 biopsies (65%), bcl-2. Group bcl-2+ patients had a lower response rate after ABVD chemotherapy - only 24 (28%) patients achieved CR or better result, as compared with 49 patients (57.6%) of the bcl-2 group. Three-year event-free survival (EFS) in bcl-2+ patients had lower 82% vs 96% in bcl-2 group (p=0.018). Multivariate analysis using the Cox proportional hazard model with the inclusion of bcl-2+, CD30 +, bcl-2+/CD30 +, age 45 and older, B-symptoms, III-IV stage, anemia, decreased serum albumin, increased LDH, leukocytosis revealed that the expression of bcl-2 on RS cells was an independent factor of poor prognosis. 3 year EFS was 52% vs 90% in bcl-2 population (p=0.022; RR=1.4). The greater relative risk was observed in a population with double expression of bcl-2 and CD30, where the 3-year EFS was 47% (p=0.012; RR=1.6).

Summary/Conclusions: The expression of bcl-2 on HRS cells can be a more powerful factor of poor prognosis than bcl-2+ cells.

PB1866

SURVIVAL ANALYSIS OF PATIENTS WITH CLASSICAL HODGKIN’S LYMPHOMA TREATED WITH ABVD: RESULTS FROM TWO REFERRAL CENTERS IN MEXICO CITY.

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Background: Classical Hodgkin’s lymphoma (cHL) is a neoplastic disease with a favorable prognosis since 85% of patients can be considered cured with current treatment strategies. Combined chemotherapy with Adriamycin, Bleomycin, Vinblastine and Dacarbazine (ABVD) has been the standard therapy for over 20 years. Epidemiological information and the regimen’s results as first-line therapy in Mexico are limited.

Aims: The aim of this study was to conduct a survival analysis in adult patients from two referral centers in Mexico City.

Methods: This is a retrospective analysis of all patients with cHL treated at the Instituto Nacional de Cancerología and the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, between 2009 and 2013. The study was approved by the local Ethics Committee.

Results: We included a total of 193 patients with a de novo diagnosis and initially treated with ABVD: 60.6% of cases were male, with a median age of 36 years (17-81 years), 71.5% were diagnosed in late clinical stages (CS). The most frequent histopathological subtypes were: nodular sclerosis and mixed cellularity (46.6% and 40.9%, respectively). The observed overall response rate (ORR) was 85.7% [Complete response (CR) = 78.2%. The RR was 90% in early CS vs 83.8% in late CS (CR rate was 84% vs 75.8%; respectively, p=0.23). Univariate analysis by logistic regression in the early CS group revealed that having a Lymphocyte:Monocyte ratio <1 presents an independent factor of poor prognosis. 3 year EFS was 83% in the group with a Lymphocyte:Monocyte ratio <1 vs 96% in the group with a Lymphocyte:Monocyte ratio >1 (p=0.023). In the group in late CS, we found that the lymphocytopenia percentage tended to favor CR [OR 1.048 (95%CI 0.994-1.105; p=0.081)] and the opposite was observed in terms of the absolute monocyte count [OR 0.999 (95%CI 0.998-1.000; p=0.082)]. Median follow-up was 35 months (0-96 months), 10.9% of cases had died at last follow-up, and median overall survival (OS) of the entire cohort had not been reached at the time of analysis (5-year OS, 87.1%). However, at the time of this analysis, the group of patients in complete remission had a greater OS than the group that did not achieve CR (p=0.0001). With Cox multivariate analysis of OS according to CS, we detected that in the group in early CS, none of the analyzed factors were significant, while in the late CS group, age >45 years was an independent risk factor [HR 6.9 (95%CI 1.80-26.60; p=0.005)] and achieving CR had a protective effect [HR 0.02 (95%CI 0.004-0.108; p=0.0001)].

Summary/Conclusions: Although OS medians had not been reached at the time of analysis, it is noteworthy that CR (84%) in early CS is lower than that reported in the literature and no related prognostic factor has been identified. The role of lymphocytes and monocytes may prove to be significant in larger series with a longer follow-up.

PB1867

OUTCOME OF PD-1 BLOCKADE IN PATIENTS WITH RELAPSED HODGKIN LYMPHOMA AND ACTIVE GRAFT-VERSUS-HOST DISEASE

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Background: Efficacy of PD-1 (programmed death-1) inhibitors in relapsed/refractory Hodgkin lymphoma (HL) has been established, but their role in relapse after allogeneic stem cell transplant (alloSCT) remains controversial due to the perceived risk of exacerbating graft-versus-host disease (GVHD). The literature is largely limited to case reports in patients with no or quiescent GVHD.

Aims: To determine the outcome of PD-1 inhibitor therapy and subsequent management in patients with concomitant biopsy proven active GVHD and progressive HL after alloSCT.

Methods: We describe the treatment and management of two patients in our centre.

Results: Case 1 had both extensive bony, lung and nodal HL with active skin, pleuropedicardial and liver GVHD 6 months after donor leucocyte infu- sion (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 partial remission with associated stable liver GVHD.

Case 2 had progressive mediastinal and pulmonary HL despite DLI-induced extensive liver and skin chronic GVHD 38 months post sibling alloSCT. Initial therapy consisted of optimisation of liver GVHD with 8 weeks of UDCA and prednisolone with improvement in liver indices (Figure 1). Pembrolizumab 25mg 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 pancycopenia and marrow hypoplasia attributed to an immune-mediated phenomenon. Despite addition of tacrolimus and increased steroids, he remains with severe liver dysfunction and pancycopenia 10 weeks after the single dose of PD1 inhibitor therapy.

Summary/Conclusions: PD-1 inhibitors can exert powerful graft vs HL effects even in patients with progression in the context of active GVHD, but at the expense of substantial GVHD exacerbation. Further exploration of approaches such as individualised dose titration according to response and 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. The red blood cell distribution width (RDW) is associated with short- and long-term outcomes of various malignancies. The prognostic value of the RDW in cHL remains unknown.

Aims: The aim of this study was to analyze the prognostic significance of RDW in cHL patients.

Methods: We retrospectively analyzed data from 54 cHL patients diagnosed from 2005 to 2016 at the University Hospital Center Osijek, Osijek, Croatia. We evaluated disease outcome, overall survival (OS) and event-free survival (EFS), and demographic, clinical and laboratory factors affecting outcome. Univariate analysis and Cox regression analysis were used.

Results: The median age of patients was 36 years, 29 were men (54%). Higher RDW levels (%) were found in patients with advanced Ann Arbor clinical stage (15.3±2.28 vs 13.12±1.3, P<0.001) and in those with poor response to therapy (15.65±3.37 (progression) vs 16.68±2.09 (partial remission), 13.95±1.82 (complete remission), P=0.008). Patients with RDW values of >14.5% (cutoff value calculated by receiver-operating characteristic) had a significantly worse two-year EFS (62.4% vs 90.4%, P=0.009) but did not differ significantly in terms of OS (P=0.2). Univariate analysis revealed that a high RDW (>14.5) was correlated with poor EFS (P=0.019). Multivariate Cox regression analysis showed that RDW >14.5% was an independent prognostic factor for EFS (hazard ratio [HR] 3.801, 95% confidence interval [CI] 1-14.45, P=0.05). The RDW allowed further borderline statistically significant risk stratification in patients who were considered to be at low risk on the basis of an International Prognostic Score less than 4 (P=0.053).

Summary/Conclusions: High baseline RDW is an independent prognostic marker of poor outcome in patients with cHL. RDW ratio is asimple, inexpensive, and independent prognostic factor for EFS that may improve the ability to identify high-risk patients with cHL. It could be an easily available and inexpensive marker for the risk stratification in patients with cHL.

PB1869

HIGH FREQUENCY OF SECONDARY MALIGNANCIES IN PATIENTS WITH LARGE GRANULAR LYMPHOCYTIC LEUKEMIA: A SINGLE INSTITUTIONAL EXPERIENCE

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Background: Large granular lymphocyte (LGL) disorders represent a spectrum of aberrant T-cell or natural killer cell lymphocytic proliferations. LGLL is classically associated with autoimmune conditions and bone marrow (BM) failure disorders. SM has been reported in association with LGLL in about 10%. Aims: The aim of this study is to evaluate the impact of SM on the clinical course of LGLL. Methods: This is a retrospective study of LGLL patients evaluated at Moffitt Cancer Center between January 1995 and May 2016. The diagnostic clinicopathological criteria consisted of LGL count > 0.5 k/μL with T-cell receptor gene rearrangement. Lower absolute number of clonal circulating LGLs with characteristic immunophenotype associated with BM involvement, cytopenias, splenomegaly and/or associated symptoms were also diagnostic. Patients with myelodysplastic syndrome were excluded. Survival analysis was performed using the Kaplan-Meier method with log-rank test. Chi-square and T-test were used to analyze association among various variables. Significant P-value was considered <0.05.

Results: Out of 668 screened patients with LGL expansions in peripheral blood, 261 met criteria for LGLL, Secondary malignancies were present in 44% (116/261) of LGLL patients, of which 38% were hematological and 80% arose prior to onset of LGLL. Most common solid secondary malignancy included skin cancer (14%), prostate cancer (12%), and breast cancer (12%), while most common hematological secondary malignancy consisted of non-Hodgkin lymphoma (17%) and chronic leukemia (14%). 5-year overall survival (OS) for all LGLL patients was 75% and 10-year OS 63%. There was a statistically significant difference in 5-year OS between LGLL patients with a secondary malignancy compared to without (p = 0.049), but no difference between both groups in median OS or 10-year OS. Patients diagnosed with a secondary malignancy prior to LGLL had worse 5-year OS (p = 0.031) and 10-year OS (p = 0.05) compared to all other LGLL patients.

Summary/Conclusions: This study showed that the frequency of a secondary malignancy is higher than previously described, especially with onset prior to diagnosis of LGLL. Even though median age of LGLL is around 60 years, it appears that age itself cannot explain this phenomenon. Our results suggest that having a secondary malignancy is a poor prognostic factor in LGLL patients.

PB1870

BENDAMUSTINE AND RITUXIMAB COMBINATION THERAPY WITH SUBSEQUENT RITUXIMAB SUPPORTING THERAPY IN RUSSIAN SUBJECTS WITH RELAPSED OR REFRACTORY INDOLENT B-CELL NON-HODGKIN LYMPHOMAS

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Background: Combination of bendamustine and rituximab has been established in many international guidelines as treatment for patients with indolent B-cell non-Hodgkin lymphoma (INHL).

Aims: Objectives of this study were to evaluate the effectiveness, safety, and tolerability of bendamustine/rituximab combination followed by rituximab maintenance therapy for relapsed or refractory (R/R) INHL patients in the Russian Federation.

Methods: Adult subjects (≥18 yr), diagnosed with R/R INHL according to local diagnostic standards, and were enrolled in this prospective observational study. Intravenous therapy was administered in 2 stages (Figure 1): a combination therapy stage followed by a rituximab supporting therapy stage for subjects who achieved complete response (CR) or partial response (PR) during the combination therapy stage. Overall response rate (ORR) was assessed after
3 (Evaluation 1) and 6–8 (Evaluation 2) 28-day cycles. Data from the full analy-
sis set (FAS) were used for the primary analysis and the per-protocol (PP) set for a subgroup analysis. Safety/tolerability was a secondary endpoint and was assessed in the safety analysis set (SAF). Response assessments used the LOCF method for substitution of missing data; overall survival (OS) and pro-
gression-free survival (PFS) were calculated using Kaplan–Meier estimates, and comorbidities were assessed using the Charlson index. The endpoint of OS was death or last contact, and the endpoint of PFS was disease progression or last contact. A sample size of 66 patients was defined assuming a P0 of 45% CR, and a P1 of 65% CR. Per protocol, the study was designed to be stopped early if 70% of patients did not reach CR or if an early termination decision was made due to adverse events. The estimated sample size was 102 patients with an expected dropout rate of 20% (n=82 patients). The primary objective is the activity of ofatumumab in terms of complete response (CR) rate. Secondary objectives include the assessment of safety, efficacy, and tolerability with a focus on quality of life (QoL) and patient-reported outcomes (PROs). The study was conducted at 70 sites worldwide, and patients were randomized to either ofatumumab 300mg or placebo up to 8 cycles. Results: Of the 102 subjects enrolled between June 2012 and October 2015, 83 subjects (52M/31F; median age 59 yr [range: 27–84]) with various NHL his-
tology: subjects with mantle cell lymphoma [n=4], diffuse large B-cell lymphoma [n=2], and follicular lymphoma transformation [n=1] were excluded from the PP population due to deviation from the INH inclusion criteria. Most study subjects were heavily pretreated with a median number of 2 prior lines of therapy before entering the study (range: 1–6). At Evaluation 2, ORR in the FAS was high (n=50; 69.9% [PP]), with 35 (42.2%) subjects achieving CR (confirmed, n=20; 24.1%; nonconfirmed, n=15 [18.1%]) and 23 (27.7%) achieving PR; ORR (defined as [CR+CR uncertain +PR]) in the PP population was 70.8% (Table 1). For FAS patients, at follow up (17 mo) neither median OS nor PFS had been reached; 2-year OS was 88.9% [95% CI: 79.7–98.9%] and 2-year PFS was 87.9% [95% CI: 80.7–95.7%]. In the SAF, 31 of 96 subjects (32.3%) reported ≥1 AE. Decreased neutrophil count, decreased white blood cell count, and infections were the most commonly reported AEs and serious AEs. Twelve deaths occurred: 5 due to disease progression (n=2) or relapse (n=3), 5 were not related to lymphoma or occurred during remission, 1 cause of death was unknown, and 1 subject died from hyperthermia and respiratory failure, which was the only death in the study considered related to combination therapy.

Figure 1.

Summary/Conclusions: Bendamustine plus rituximab therapy followed by rit-
ximumab maintenance therapy was generally well tolerated and demonstrated clinical effectiveness in Russian R/R patients with INHLs. Although a number of subjects with aggressive lymphomas were included in the FAS, the ORR rate was not considerably different from the PP population (ORR: 69.9% [FAS] vs 70.8% [PP]).
continue to the planned 15 patients (interim analysis). Here we present safety analysis results.

Results: Ten patients (6 males, 4 females; median age: 69.5 years, 9 ≥65 years, 1 <65 years) were analyzed for safety. Eight patients were previously treated with rituximab, 26 adverse events (AEs) occurred in 7 patients, with only 5 grade 3-4 AEs. Ten AEs were drug-related, 30% were of grade 3 (Table 1). Three SAEs occurred: hypersensitivity, n=2, both related, asynaptic, n=1, unrelated to study drug. No AEs leading to treatment withdrawal were reported and no patients died on study. Hematological and biochemical abnormalities included: neutropenia (any grade 6 cases, grade 3-4: 4), thrombocytopenia (grade 1-2: 3 cases), lymphopenia (grade 1-2: 2 cases), leukopenia (grade 1-2: 5 cases), 1 case of GGT (grade 3, at baseline grade 2), 9 cases of ALP increase (all grade 1), 1 case each of AST, ALT and bilirubin increase (all grade 1). Preliminary response assessment in these 10 patients documented 5 CR, 4 Partial Responses (PR) and one patient with progressive disease (PD) at the end of treatment.

Table 1: List of AEs.

<table>
<thead>
<tr>
<th>Drug-related AEs</th>
<th>N of patients (any grade 2-4)</th>
<th>Non-drug related AEs</th>
<th>N of patients (any grade 2-4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutropenia</td>
<td>6 (2)</td>
<td>Anemia</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>2 (2)</td>
<td>Neutropenia</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1 (0)</td>
<td>Rheumatoid arthritis</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1 (0)</td>
<td>Dyspnea</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>1 (0)</td>
<td>Pancytopenia</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Bone pain</td>
<td>1 (0)</td>
<td>Gout</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (0)</td>
<td>Arthralgia</td>
<td>1 (0)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Ofatumumab is safe and generally well-tolerated even in elderly patients with R/R SMZL. No cases of unexpected adverse drug reactions were documented. In a series of patients largely pre-treated with rituximab, ofatumumab resulted in a 90% overall response rate, 50% being CR. Complete results of the interim analysis will be presented at meeting.

PB1873
TREATMENT PATTERNS AND RESPONSE TREATMENT IN PATIENTS WITH FOLLICULAR LYMPHOMA IN ROUTINE CLINICAL CARE – A UNITED STATES ELECTRONIC MEDICAL RECORD DATABASE STUDY
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Background: FL represents 70% of all indolent non-Hodgkin lymphomas, and it is widely recognized that FL is a heterogeneous disease, with patients presenting with differing amounts of tumor burden and prognostic indicators. The NCCN guideline recommends using rituximab as a single agent or in combination with other chemotherapies as first-line therapy (1LT) or second-line therapy (2LT). No recommendations are provided beyond 2LT.

Aims: To evaluate treatment patterns and associated response in patients with newly diagnosed FL in routine care in the US.

Methods: Newly diagnosed FL patients aged ≥18 years were selected from Humedica, a large, national US EMR database, between 01/01/08 and 07/31/15 if they had ≥1 visit recorded in the electronic chart of FL diagnosis codes. The date of the first FL record was the index date. Patients were followed from index until end of continuous activity, progression to diffuse large B-cell lymphoma (DLBCL), death, or end of study period (09/30/15) and were evaluated for FL treatment patterns and treatment response. Possible remission was defined as no additional chemotherapy and no supportive care. The end of treatment was defined as the receipt of supportive care <30 days after end 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 before end of LOT for <30 days. Remission was defined as complete remission, partial remission, and stable disease.

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%). Ofatumumab plus 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 with available data after 1LT, 41.3% (n=83) had evidence of remission, 35.4% (n=71) progressed, and 1.5% (n=3) had no evidence of remission. 165 patients who progressed after 1LT received second-line therapy (2LT); 35% received a 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 progressed after 1LT, 11% (n=9) had evidence of remission, 8% (n=7) progressed, and 0% (n=0) had no evidence of remission. 8 patients who progressed after 2LT received third-line therapy (3LT); 35% received a single agent, while 64.4% received combination chemotherapy. In 3LT, rituximab (11.1%) was the most common single agent: bendamustine+rituximab (20.0%) and rituximab (32.0%) 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 patterns 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. This fact is one factor considered in the FLIPI-1 and FLIPI-2 prognostic index. Positron emission tomography/computed tomography (PET-CT) is a noninvasive technique that shows high sensitivity of detecting nodal and extranodal lymphoma involvement, specially in aggressive subtypes. Some studies have described a high specificity (92-100%) in the detection of bone marrow involvement in aggressive NHL. However, its role in low-grade indolent lymphoma such as follicular lymphoma remains controversial.

Aims: To analyze retrospectively the diagnostic accuracy of PET-CT in comparison to BMB in the initial staging of new FL in a single centre in daily practice.

Methods: One hundred and thirty-six patients with de novo FL have been diagnosed in our institution from June 2005 to October 2016. Of them, 64 who underwent both BMB and PET-CT before treatment were evaluated. The BMB was evaluated by hematologist-pathologist and the interpretation of PET-CT images was interpreted by a nuclear radiologist. Positive BMB was defined as the presence of CD20 +,CD10 + or Bcl-2 + lymphoid infiltration. No molecular biology techniques were done in the bone marrow tissue. PET-CT bone marrow involvement was defined as an elevated FDG uptake in the bone marrow than those in liver or mediastinum.

Results: Thirty-five male and 29 female were included. The median age at diagnosis: 58 years (range 23-84). Seventy-four patients had grade 1-2 FL and 30 grade 3a FL. Bone marrow involvement was diagnosed in 33 of 64 patients (51.1%) by BMB. Out of the 17 patients with positive PET-CT, 4 had negative BMB. Out of 33 patients with positive BMB, 13 had a positive PET-CT (Table 1). The sensitivity and specificity of PET-CT was 39% and 87%, respectively. The positive predictive value and negative predictive value was 76.5% and 57%, respectively.

Table 1. Detection of BMO involvement: BMB and PET-CT results.

<table>
<thead>
<tr>
<th></th>
<th>BMB</th>
<th>PET</th>
<th>TOTAL</th>
</tr>
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<tbody>
<tr>
<td>-</td>
<td>27</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>+</td>
<td>12</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>TOTAL</td>
<td>39</td>
<td>41</td>
<td>80</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Our study shows a very low sensitivity of PET-CT in the daily practice. These results contrast with those reported in some recent studies in aggressive lymphoma. However, the high positive predictive value raises the question about the usefulness of BMB in these PET-CT positive cases. In our opinion, with the current data, BMB should be performed in indolent NHL patients.

PB1875
SURVIVAL OUTCOMES AFTER FIRST-LINE THERAPY IN FOLLICULAR LYMPHOMA USING A UNITED STATES ELECTRONIC MEDICAL RECORD-BASED COHORT
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1Millennium Pharmaceuticals, Inc., a wholly owned subsidiary of Takeda Phar-
Background: FL is a heterogeneous disease, and clinical presentation is highly variable. The Follicular Lymphoma International Prognostic Index (FLIPI-2) identifies prognostic factors at diagnosis but does not predict in whom and when to initiate first-line therapy (1LT). 1 Recommended therapies for 1LT vary by stage, symptomatology, and tumor burden but include monotherapy with rituximab (R) or in combination with other chemotherapies. Survival of FL patients in the R era has greatly improved, but few studies have evaluated survival outcomes in patients seen in routine clinical care.

Aims: This study aimed to evaluate survival outcomes in a US population of newly diagnosed FL patients seen in routine clinical care.

Methods: A retrospective study was conducted in which the presence of ≥1 inpatient record or ≥2 outpatient records with FL diagnosis codes were used to identify newly diagnosed FL patients from Humedica, a large US EMR database, between 01/01/08 and 07/31/15. The study index date was the first FL record. Patients who subsequently initiated 1LT for FL were followed from the date of treatment initiation until death, loss to follow-up, or end of study (09/30/15) for the evaluation of the survival outcomes. Median progression-free survival (PFS) (defined as initiation of second-line therapy, evidence of supportive care ≥30 days after the end of a line of therapy, or death), median overall survival (OS), and PFS and OS rates at 2 years following initiation of 1LT were evaluated using Kaplan-Meier analyses.

Results: 1,346 newly diagnosed FL patients who initiated 1LT met the patient selection criteria. 47.7% were male, and the mean age was 65.4 years (SD: 12.7). At baseline, 16.6% of patients had a Charlson Comorbidity Index of ≥2, and the most common comorbidities were diabetes (14.5%) and chronic pulmonary disease (11.2%). 1LT consisted of both monotherapy (38.6%) and combination therapy (61.4%). For monotherapy, R was the predominant agent used (85.1%); for combination therapy, bendamustine+R (43.8%) and R-CHOP (24.6%) were the most common. Kaplan-Meier analysis revealed that the 2-year OS and PFS rates (from initiation of 1LT) were 86.9% and 64.6%, respectively. Median OS was not reached, and median PFS was 48.1 months (95% confidence interval: 39.4, 58.4).

Summary/Conclusions: The 2-year OS and PFS rates in this newly diagnosed FL patient cohort who received 1LT (the majority of which was R-based) were consistent with expectations in a post-R era. Future analysis will explore the differences in clinical characteristics and survival outcomes for patients who received R monotherapy versus 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

Background: The role of F-18 FDG-PET/CT for the detection of bone marrow involvement in the staging of newly diagnosed patients with lymphoma was reviewed in the Recommendations of Lugano Classification. They conclude that a positive PET/CT should trigger bone marrow biopsy (BMB) in patients with bone marrow involvement by biopsy, 5 patients had BMPET- and PET/CT detected bone marrow involvement in all of them.

Aims: We study the value of F-18 FDG-PET/CT for the detection of bone marrow involvement in the initial staging of patients with lymphoma.

Methods: Newly diagnosed patients with HL, DLBCL and FL who underwent F-18 FDG PET/CT and bone marrow biopsy for initial staging between January 2007 and June 2016 were included. We analyze specificity, sensitivity and concordance of PET/CT compared with bone marrow biopsy. In discordant cases, we review if there was any difference in the staging.

Results: 161 patients were included, 69 DLBCL (38 male, 31 female, median age 59 years), 44 HL (24 male, 20 female, med32y), 48 FL (23 male, 25 female, med55y). 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. Two patients of HL had BMB+ and PET/CT only detected bone marrow involvement in 1 patient.

Summary/Conclusions: Our series confirms that PET/CT is useful to detect bone marrow involvement in the initial staging of Hodgkin Lymphoma and DLBCL. PET/CT avoids 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 invasion by biopsy. These results support the histological assessment of bone marrow in the initial staging of follicular lymphoma.
**PB1880**

**PREDICTIVE FACTORS FOR INFECTIOUS ADVERSE EVENTS IN PATIENTS WITH B-CELL NON-HODGKIN LYMPHOMA TREATED WITH BENDAMUSTINE-RITUXIMAB (R): RISK MAINTENANCE. RESULTS OF A RETROSPECTIVE STUDY WITH A LONG PERIOD OF OBSERVATION**

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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 cycles of BR maintenance between 2010 and 2016 at our institution. The AEs – including neutropenia (N), neutropenic fever (NF), lymphopenia, infections episodes and the occurrence of second tumors - were recorded according to the CTCAE v4.0 grade score. We compared the patients who were treated until a second infection was recorded with the ones that did not. Univariate analysis with Fisher’s exact test was used to evaluate the potential risk factors.

**Results:** The median age at the first treatment cycle was 66 years (range 36-89), 33 patients (50%) were ≥65 years, 27 (41%) were male, 53 (82%) had advanced disease and 37 (60%) had bone marrow involvement. Thirty (46%) patients had follicular lymphoma, 17 (26%) mantle cell lymphoma, 11 (17%) marginal lymphoma, 5 (7%) diffuse large B-cell lymphoma and 4% other indolent lymphomas. Thirty two patients (49%) received BR as first line treatment, 51% as second line and above. Bendamustine was administered either at the dosage of 90 or 100 mg/m² on days 1, 2 and R was administered at a dose of 375 mg/m² iv or sc, on day 1. Therapy was administered every 4 weeks up to 6 courses. Twenty nine patients (46%) received R maintenance every 4-12 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 6/22 patients, varicella zoster virus infection in 4/22, cytomegalovirus reactivation in 2/22 and other infections in 10 patients. At univariate analysis, the infectious AEs were associated only with lymphopenia during the second cycle (p=0.043) and with neutropenia during the second, third and fourth cycle (p=0.026, p=0.003, p=0.018, respectively). No correlation with age, line of treatment and G-CSF administration was documented. Other AEs were: grade 3/4 neutropenia (41%), grade 3/4 lymphopenia (80%). We reported also a 5% incidence of second tumors after treatment (lung cancer in 2 patients and prostate cancer in 1).

**Summary/Conclusions:** In our analysis, BR/R maintenance confirms a toxicity profile similar to that reported in previous experiences. According to our results, an early lymphopenia and neutropenia (after two cycles) are predictive factors for infections AEs and for premature treatment discontinuation. Twenty % of patients discontinued treatment mostly because of the early withdrawal due to infectious complications. These data raise the question on the role of antibacterial, antiviral and primary G-CSF prophylaxis in all patients treated with BR.

**PB1882**

**CINCOLENT CHARACTERSISTICS AND PROGNOSIS OF PATIENTS WITH INDOLENT NON-HODGKIN LYMPHOMA AND RISK OF TRANSFORMATION TO AGGRESSIVE LYMPHOMA: A SINGLE JORDANIAN CENTER EXPERIENCE**

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**Background:** Indolent Non Hodgkin Lymphomas (INHL) are slow growing lymphomas that usually arise from B-Cells. They are characterized by slow appearance and progression of symptoms compared to aggressive non Hodgkin lymphoma (NHL) namely Diffuse large B-cell Lymphoma (DLBL). Small percentage of INHL might transform to aggressive NHL.

**Aims:** We aim to describe the clinical characteristics, prognosis and risk of transformation to aggressive lymphoma in patients with INHL in North Jordan as a model for other Middle East countries in which such data is lacking.

**Methods:** All patients diagnosed with INHL between Jan 2003 to Jan 2017 were retrospectively reviewed. Clinical and laboratory data at time of diagnosis including gender, age, lactate dehydrogenase level (LDH), pathological subtype, sites involvement, PET/CT scan were studied. Extramedullar involvement was confirmed either by histopathological studies or CT and PET/CT scan. Transformation to aggressive lymphoma was confirmed by histopathological studies. Patients were followed and overall survival rate was calculated. Mean survival times were calculated using Kaplan-Meier method.

**Results:** Among 265 patients diagnosed with 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 / chronic lymphocytic leukemia (CLL), 20 patients (22.7%) had follicular lymphoma (FL), 15 patients (17%) had marginal zone lymphoma (MZL), 6 patients (6.8%) had mantel cell lymphoma (MCL) and 2 patients (.78%) had unspecified NHL. Mean age of MZL (53.2 years) and FL (55.3 years) were significantly lower than mean age of MCL (58 years) and CLL (62.77 years). 22 patients (23.9%) had extra nodal involvement. There was no significant association between INHL subtypes and extra nodal sites involvement. (P-value=.001). 60% of patients with MZL, 50% of patients with MCL, 20% of patients with FL and 8.5% 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. INHL subtypes and extra nodal sites involvement. (P-value=.0004) There was no significant association between mean age and mean survival rate of patients with 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=.0004).

**Summary/Conclusions:** Prevalence of INHL among patients with NHL in North Jordan is 33.2%. The most common INHL subtypes in our patients were Large B-cell Lymphoma (DLBL). There was significant association between INHL subtypes and transformation to DLBL. INHL subtypes and extra nodal sites involvement.
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 patient’s co-morbidities, risk of visual impairment, need for systemic vs. local therapy, histological diagnosis and anticipated side effects. As treatments are so effective the long term consequences and possible late effects need to be acknowledged and avoided if at all possible. Observation is an acceptable approach in asymptomatic patients when there is no immediate risk of visual impairment. Radiotherapy is an effective first line treatment in symptomatic disease or 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 risk stratify indolent subtypes, but criteria do not include age. Here we present our single center analysis of clinical-pathological 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 subtypes. This is a retrospective study of patients treated at Moffitt Cancer Center between January 1990 and December 2016. Patients were identified using our PCBCL database and diagnosis was verified by independent hematopathologists and dermatopathologists. Staging was determined according to ISCL/EORTC recommendations. Demographics, lymphoma subtype, stage, disease course, and CLIPi scores were recorded. 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 between 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% PCDLBCL, 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 (PCDLBCL) and low grade (indolent) subtypes. PFS according to stage in indolent subtypes showed a statistically significant difference in PFS among the strata.

Summary/Conclusions: We found that age is a highly statistically significant prognostic parameter in PCBCL, as patients ≤60 years had a longer PFS in comparison 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 MCL (41 patients, 73.2%), followed by marginal zone lymphoma (17 patients, 30.4%), and 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 antigentic stimulus in 11 patients (Helmocobacter pylori, Sjogren’s syndrome, Hashimoto’s thyroiditis). The molecular study of MALT1 was performed in 25 patients and 3 presented the translocation (12%). Six of them had no signs of lymphoma. Antinuclear antibodies were positive in 15 of 32 patients (46,9%). Monocytopenia was detected in 71 patients (82.1%) after the first line of treatment (WHO (2016 revision). In addition, we performed an autoimmunity test in most 50% of the patients had B-RAF 464-469 codon mutations (One B-RAFG464E, one B-RAFG466E, one B-RAFG469E) Two patients were positive due to febrile neutropenia. 43% received cotrimoxazole prophylaxis and 3 opportunistic infections were recorded (1 patient was diagnosed and treated due to  Mantle cell lymphoma (MCL) a year ago and found in remission for both MCL and HCL; one was treated with one cycle of cladribine therapy in our department.

**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% of non-Hodgkin lymphomas, more commonly seen in man. Diagnosis is based on the examination of peripheral blood smear, flow cytometry and the bone marrow aspiration-biopsy. Recently, B-RAFV600E mutation was demonstrated in 10% of Tiacci HCL case series.

**Aims:** Our study is to investigate the frequency of B-RAFV600E mutation and other rare mutations of B-RAF (B-RAF G464E, B-RAF G466E, B-RAF G465V) and their relation with clinical data and treatment responses.

**Methods:** Charts of 13 patients diagnosed with HCL were analyzed retrospectively. Patients’ clinical parameters were evaluated. HCL variant type patients were excluded. Paraffin blocks of spleen or bone marrow tissues are obtained from the pathology archives. One thin section (10 micron) of bone marrow or three sections of spleen are cut and DNA extracted by spin column technique using DNA extraction kit. (QiAmp DNA FFPE Tissue Kit, Qiagen) After specrophotometric measurement of DNA; common and uncommon mutations of B-RAF were investigated. (Qiagen PyroMark Q24 system, Therascreen BRAF V600E Pyrokit 24, V1 (1/2) kit) Mutation and clinical data analysis were conducted using the SPSS 15.0 software. The study was approved by the local ethics board of Dokuz Eylul University.

**Results:** Male/female ratio was 9/4. Male 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 Male/female ratio was 9/4. Median age at diagnosis was 48(37-87). ECOG≤ 2 in 95% of cases, 73.2% in stages III-IV and FLIPI ≥3 in 48%. Bulky mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow infiltration in 80% of patients. Among the patients who received a complete remission (CR) 76.1% and 10 partial remission (PR) in 39% of patients treated with immunochemotherapy (37%), 10 immunotherapy (21.7%), 8 surgery (17.4%) and 3 progressions (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.

**Methods:** Retrospective analysis of patients with relapsed FL treated with BR in 7 spanish hospitals on behalf of the Spanish Lymphoma Group (GELTAMO). The study was approved by the reference Ethnic Committee and by all of the participating centres. All patients acceded to the treatment through the compassionate use program.

**Results:** 41 patients were valid for analysis. Characteristics: 70% males with a mean age of 62 years (30-87). ECOGs ≤2 in 93% of cases, 73% in stages III-IV and FLIPI ≤2 in 48%. Bulky mass in 13% of patients, LDH and β2-microglobulin increased by 12% and 41.2% respectively and bone marrow infiltration in 80% of patients. Among the patients who received a complete remission (CR) 76.1% and 10 partial remission (PR) in 39% of patients treated with immunochemotherapy (37%), 10 immunotherapy (21.7%), 8 surgery (17.4%) and 3 progressions (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.

**PB1888**

**USE OF RADIATION THERAPY FOR THE TREATMENT OF GASTRIC MALT LYMPHOMA**

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**Background:** Gastric mucosa-associated lymphoid tissue (MALT) lymphoma is a rare disease however, the incidence is increasing and closely associated with helicobacter pylori (HP) infection. One choice of treatment of gastric MALT lymphoma refractory to HP sterilization is radiotherapy.

**Aims:** Our aim was to analyze the response to treatment with definitive radiotherapy in our department.

**Results:** Between January 2014 and January 2017, 8 patients with gastric MALT lymphoma were treated with eradication therapy of HP, followed by definitive radiotherapy. The average total dose was of 38 Gy to the stomach in a once-daily schedule. Follow-up included computed tomography scan and
endoscopy with biopsies at regular intervals. The median follow-up was 14 months.

Results: In all patients we got complete responses (CR) with no tumor detectable by endoscopy or biopsy after initial treatment, but after 2 years one of them relapsed and required immunochemotherapy. The most common acute toxicities were fatigue and nausea, in our patients. In any case late toxicities were observed. The overall survival was 100% after 2 years.

Summary/Conclusions: In selected patients who are not responsive to HP sterilization, definitive radiotherapy can be an efficient therapy with tolerable complications, preservation of stomach and sustained response over time.

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±standard deviation of lymphoma patients was 61.6±15.6 years and 56.6% were male. For the majority of patients (81.1%), intended use of lipegfilgrastim was in PP. Exposure to lipegfilgrastim has been documented for 228 patients with an average of 4.76 cycles per patient. Data on CT dose modifications and neutropenic events following the first lipegfilgrastim-supported cycle were available for 144 and 167 patients, respectively. CT dose was never omitted. CT dose delays were observed in 8.0% (PP) and 18.8% (SP) of patients and CT dose reductions in 4.5% (PP) and 12.5% (SP) of patients. In the first lipegfilgrastim-supported cycle, febrile neutropenia was recorded in 4.5% (PP) and 3.0% (SP) of patients; severe neutropenia was recorded in 7.5% (PP) and 9.1% (SP) of patients. Throughout the treatment, 22 (9.6%) patients exposed to lipegfilgrastim reported at least 1 adverse drug reaction (ADR). The most common ADRs were myalgia and musculoskeletal pain. Serious ADRs were reported by 11 (4.8%) patients.

Summary/Conclusions: Lipegfilgrastim is effective and well tolerated in the real-world setting in lymphoma patients, administered either in PP or SP. The results suggest that lipegfilgrastim administered in PP might give better outcomes in terms of dose delays and dose reductions than when administered in SP.

PB1890

TUBERCULOSIS IN ACUTE LEUKEMIA- AN ANALYSIS OF CLINICAL CHARACTERISTICS AND IMPACT ON MANAGEMENT IN 25 PATIENTS

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Background: Patients with acute leukemia represent an immune-compromised population, with innate, humoral and cellular immune 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-tubercular therapy was conducted.

Results: A total of 25 patients with acute leukemia were identified who were also diagnosed with tuberculosis. 10 patients had Acute Myeloid Leukemia, 7 had Acute Promyelocytic Leukemia, 5 had Acute Lymphoblastic Leukemia, 2 had Mixed Phenotypic Leukemia while 1 had Myeloid Sarcoma. The mean interval between diagnosis of tuberculosis and acute leukemia was 37.2 weeks, with 2 patients being diagnosed after completion of therapy of acute leukemia.
and one patient was diagnosed post mortem. The most common organ involved was the lung, which was seen in 80% of patients and 20% of patients had disseminated tuberculosis. The development of tubercular infection led to alteration of therapy for the acute leukemia in 24% of cases, while it was postponed in 44% of cases. In particular, hypomethylating agents were used successfully in two patients with AML as bridge therapy to high dose chemotherapy. 76% of patients were cured of tuberculous infecions with therapy, while 1 patient expired due to tuberculosis and 3 patients could not receive adequate therapy for tuberculosis. 3 patients went on to undergo HSCT post treatment for tuberculosis, and none had a flare of the disease post transplant.

Summary/Conclusions: The presence of tuberculosis infection in patients of acute leukemia has an impact on the overall management of the patient and strategies such as utilization of hypomethylating agents as bridge therapy may help in successful management of the leukemia. A high index of suspicion is required to suspect and diagnose the presence of tuberculosis as the manifestations are more commonly attributed to fungal infections or to the leukemia per se. Patients usually have a febrile episode with bacteremia and the presence of tuberculosis infection does not forego treatment options such as HSCT or high dose chemotherapy for these patients.

PB1891
INCIDENCE OF BACTEREMIA BY MULTI-RESISTANT BACTERIA IN HEMATOLOGY PATIENTS. A DESCRIPTIVE EPIDEMIOLOGIC STUDY FROM A THIRD LEVEL HOSPITAL
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Background: In recent years the incidence of multi-resistant bacteria (MRB) infections have notably increase. These infections are especially serious in hematological patients because of the immunosupression derived from their illness and their treatments. This increase is related to a high mortality rate and high health costs due to the severity of the infections and the difficulty in setting adequate therapy due to the lack of new antibiotics against these pathogens.

Aims: Define the MRB infections incidence and ways of presentation. As a secondary goal we try to determine if the isolation of these MRB has affected our empiric antibiotic therapy decision.

Methods: We retrospectively collected all positive blood stream cultures from hematologic patients from January 2012 to December 2016. We studied the characteristics, clinical features and pathogen isolates of our patients when the blood cultures were obtained.

Results: 1005 positive blood stream cultures were collected in 382 patients. The main characteristics of the patients are shown on Table 1.

Table 1.

<table>
<thead>
<tr>
<th>Pathogen Isolated</th>
<th>Number of Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>120</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>80</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>50</td>
</tr>
<tr>
<td>S. aureus</td>
<td>40</td>
</tr>
</tbody>
</table>

The infection source was: central venous catheter (CVC) in 48% of patients (including tunnelled 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 (Table 2). BMR infections increased significantly in last year, mainly associated to CP, 0.5% in 2012 up to 7.1% in 2016 (Figure 1). 29.5% of MRB infections developed in patients with serious illness at diagnosis.

Summary/Conclusions: - Current antimicrobial resistance, especially concerning G- in our study, is particularly worrisome due to development of resistance to all available antimicrobial agents. The incidence of multi-resistant G+ is not very high. - Clinical presentation in MRB infections is more serious in our experience, and the mortality doubles in relation to the difficulty to establish appropriate treatment. - Severity sings at infection diagnosis in MRB carriers had led us to a change of empirical antibiotic therapy. - As reported in previous literature, prevention of transmission, a quick establishment of diagnosis and an effective treatment, along with a correct and limited use of antibiotic therapy could decrease the development of MRB.

PB1892
INFECTIONS PRESENTING IN THE BONE MARROW IN HIV POSITIVE PATIENTS AND THEIR MORPHOLOGICAL ASSOCIATIONS – SIX YEAR DATA FROM AN INDIAN TERTIARY CARE HOSPITAL
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Background: Centre of Disease Control enlists more than 20 infections considered as Acquired Immunodeficiency Syndrome(AIDS) defining. Progression of the disease and falling CD4 counts are the most important risk factors in acquiring these infections. Most of the cases present with non specific symptoms including fever, respiratory and gastrointestinal manifestations . A bone marrow examination is helpful in establishing the diagnosis in many of these cases.

Aims: The aim of this study was to evaluate the incidence of marrow infections in HIV positive patients and to study their morphological spectrum.

Methods: This is a six year retrospective study carried out in a tertiary care hospital in North India. All bone marrow aspirates and biopsies from HIV positive patients were retrieved and evaluated for the presence of infectious etiologies. Cytocchemical stains like Acid Fast Bacillus, Periodic Acid Schiff , Gomori Methenamine Silver and Mucicarmine were performed wherever needed. The associated morphological features which may assist diagnosis were noted.

Results: Bone marrow samples (either aspirates or biopsies or both) were available in 185 HIV patients. Out of these, fifty three cases (33.5%) were associated with infections. The most common infection in these patients was Mycobacterium Tuberculosis (22.7%). In addition, five cases of Histoplasmosis (2.7%), two cases of Cryptococcosis, two cases of Leishmania donovani, and one case of Plasmodium falciparum, Parvovirus and Microfilaria each were noticed. The morphological spectrum associated with infections in these cases included lymphoplasmacytic infiltrate(68%), granulomas(66%), macrophage infiltration, hemophagocytosis, gelatinous marrow transformation and marrow hypoplasia. Two cases of M tuberculosis were associated with maturation arrest in the bone marrow. One case of Tuberculosis was associated with Non Hodgkin’s Lymphoma. Myelodysplasia was seen in association with Leishmania infection.
Summary/Conclusions: A wide spectrum of infections may be observed in HIV positive patients in the bone marrow. Bone marrow aspirate and biopsy are essential, rapid and cost effective techniques to arrive at the right diagnosis in such cases. Features like hypoplasia, myelodysplasia and maturation arrest may be attributable to infections.

PB1893
UTILITY OF BONE MARROW BIOPSY IN FEVER OF UNKNOWN ORIGIN: A CRITICAL ANALYSIS OF A RETROSPECTIVE SERIE
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Background: The utility of bone marrow biopsy trephine (BMT) as a diagnostic tool in patients with fever of unknown origin (FUO) is a subject of controversy and debate. BMT has been shown to be safe and useful in patients with HIV/AIDS but its value in immunocompetent patients has not been sufficiently assessed. It’s reported the use of diagnostic BMT as a rapid decision-making tool in patients with FUO showed a high diagnostic yield, but BMT offers faster results. Thus, this procedure assists in clinical decision-making and the refinement of treatment in a more timely manner.

Aims: To analyse the outcome of pediatric cancer patients that result in admissions to the intensive care unit (ICU).

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%) the histology revealed the presence of granuloma and/or lymphohistiocytic aggregates; one secondary hemophagocytosis (3.2%) and one mastocytosis infiltrate (3.2%). Six patients had a previous diagnosis of HIV/AIDS (19%). Sub analysis in HIV/AIDS patients revealed positive BMT culture in 2 of the patients (6.4%). Cultures demonstrated Mycobacterium tuberculosis and Mycobacterium avium intracellularuris. There was one case in which a pathogen was grown in culture but that had a negative of ‘direct examination’. The associations most likely related factor to contribute to the diagnosis in HIV/AIDS was male predominance (58% odds ratio [OR] 2.95; 95 CI, 1.19-4.25), clinical lymphadenopathy (OR 4.97; 95 CI, 1.90-2.44) or anemia (OR, 2.21; 95 CI, 1.26-3.84). Reactive myeloid hyperplasia was represented 15 cases (48%). Non- hematological diagnostic (lymphoma, Leukemia) was made on the exclusive bases of biopsy results.

Summary/Conclusions: Bone marrow examination is an integral part of investigation of FUO, however, morphological finding alone would not be sufficient to ascertain the diagnosis. In present study only two cases of established infections were identified. Both were present in HIV/AIDS. These results are explained because a highly active antiretroviral therapy has reduced incidence of opportunistic infections. The percent of opportunistic infections diagnosed by BMT was very low and did not justify an invasive procedure. The presence of granulomas in trephine biopsy increases the likelihood of an aetiologic diagnosis in these patients. Bone marrow biopsy is still a useful ancillary procedure for establishing the diagnosis of FUO, only if used in the adequate context.

PB1894
THE OUTCOME OF PEDIATRIC CANCER PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT OF A TERTIARY HOSPITAL IN GWANGJU-CHONNAM, KOREA
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Background: Recent advances in supportive care have considerably improved the prognosis of pediatric cancer patients. However, the use of aggressive cancer treatment is also associated with complications and life-threatening events that result in admissions to the intensive care unit (ICU).

Aims: This study aimed to analyze the outcome of pediatric cancer patients admitted to the ICU.

Methods: A retrospective analysis of 84 ICU admissions of cancer patients <21 years old between May, 2004 and Aug, 2016 at Chonnam National University Hwasun Hospital (CNUNH) was undertaken. The risk factors for short-term outcome (survival at the time of discharge from the ICU) were analyzed. After excluding scheduled perioperative admissions, the records of 81 admissions (75 patients) were reviewed.

Results: Hematologic cancer patients represented 71.6% of admissions. The mortality for individual indication was as follows: bleeding, 66.7%; respiratory failure, 59.4%; systemic infection 57.5%, anterior mediastinal syndrome, 50%, neurologic disorders, 37.5%, renal disorder, 37.5%, and so on. ICU mortality after hematopoetic stem cell transplantation was 66.7%, mostly within 100 days post-transplant. The median Pediatric Risk of Mortality Score (PRISM) III score of survivors was lower than that of non-survivors (11.3±5.1 vs 19.9±10.9; P<0.001). The mortality rates were 70.3% and 27.3% in patients with high (>15 points) and low (<15 points) PRISM III score, respectively (P<0.001). Mortality rate was significantly related to the presence and number of organ system dysfunction (P<0.01 and P<0.001, respectively), positive inotropic support (P<0.01), and mechanical ventilation (P<0.001). By using multivariate logistic regressions, the independent risk factors were mechanical ventilation (OR, 8.0; 95% CI, 1.7-21.3; P<0.01), and 33 organ system dysfunction (OR, 18.5; 95% CI, 4.4-77.0; P<0.001). Hematologic cancer patients had higher mean PRISM3 score (16.5±4 vs 12.2±8.6; P=0.51) and higher risk of sepsis (39.3% vs 13.0%; P<0.05) as 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 this population.

PB1895
EFFICACY AND SAFETY OF TIGECYCLINE IN FEBRILE NEUTROPENIC PATIENTS WITH HEMATOLOGIC MALIGNANCIES AND CARBAPENEM RESISTANCE: A MULTICENTRE RETROSPECTIVE STUDY FROM CHINESE PEOPLE
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1The First Affiliated Hospital of Xiamen University, Xiamen, 2Nanfang Hospital, Southern Medical University, Guangzhou, 3Huizhou Municipal Central Hospital, huizhou, 4Nanfang Hospital, Southern Medical University, Xiamen, China

Background: Tigecycline has broad spectrum activity against multidrug-resistant (MDR) bacteria, but few investigations of tigecycline in febrile neutropenic (FN) patients with malignancy are available.

Aims: This study attempts to investigate the efficacy and safety of tigecycline in FN and carbapenem resistant patients with hematologic malignancies.

Methods: The study of 109 patients with hematologic diseases and FN were retrospectively analyzed. They are unresponsive to carbapenems for 3~5 days before receiving tigecycline (loading dose 100 mg; then 50 mg every 12 hours). Clinical response to treatment was defined as clinical cure, improvement or failure. Meanwhile, the adverse events were documented.

Results: The 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 hypothesisical 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 0.1×109/L, the clinical effective rate was 76.9% (61.3% 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.

PB1896
BONE MARROW CYTOLOGICAL CHARACTERIZATION OF PATIENTS WITH HIPERREACTIVE MALARIAL SPLENOMEGALY
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Background: Hyperreactive malarial splenomegaly (HMS) is a common cause of massive splenomegaly in malarial-endemic areas. At present, diagnosis of patients with suspected HMS in tropical medicine departments of European hospitals is relatively frequent due to immigration and the return of missionaries and NGO workers after long periods in tropical countries. Diagnostic protocols for HMS usually include a cytological study of bone marrow, because clinical similarities between HMS and lymphoproliferative disorders have been reported. However, there are no large series in the literature that estimate a bone marrow cytological standard associated to HMS. Another important issue is that patients with HMS are often multiinfected by other pathogens. Therefore, there are no diagnostic criteria for HMS.

Aims: The aim is to define the bone marrow cytological pattern of patients with confirmed HMS, as well as of HMS patients with associated viral (HIV, HBV, HCV) or parasitic diseases.

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Methods: A retrospective cytological study of bone marrow aspirates from 95 patients with HMS (n=27), HMS+HIV (n=8), HMS+HCV/HBV (n=11) and HMS+intestinal parasitosis (n=49) has been performed.

Results: Bone marrow cellularity was normal in all the groups studied except in HMS+HIV patients, in which the cellularity was very diminished (statistically significant difference, p<0.01). Most frequent alterations observed in all samples (HMS and HMS+other entities) that could define the HMS-bone marrow cytological pattern, were: - Erythroid hyperplasia with dyserythropoiesis, which is reflected in a decreased myeloid-erythroid ratio. - Increased eosinophils percentage. - Increased lymphocytes percentage. - Increased plasma cells percentage and detection of Mott cells in a significant proportion of samples from all series (48.1% of HMS samples). Quantitative results for these variables are summarized in Table 1. Lymphocytosis was significantly increased in HMS+HIV/HCV bone marrow (p<0.04). Significant detection of atypical lymphocytes (>4%) varied widely between the groups, ranging from 14.8% of HMS bone marrows to 75.0% of HMS+HIV bone marrows (statistically significant difference, p<0.01).

There was no lymphoid evidence in any case. No quantitative or qualitative alterations were detected in megakaryocytes, except for a slight decrease in HMS+HIV bone marrows (statistically non-significant difference) (Figure 1).

Table 1. Quantitative results (mean±standard deviation).

<table>
<thead>
<tr>
<th>Reference values</th>
<th>HMS</th>
<th>HMS+HIV</th>
<th>HMS+HCV</th>
<th>HMS+HBV</th>
<th>HMS+HIV/HCV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloid-erythroid ratio</td>
<td>0.31</td>
<td>0.20±0.8</td>
<td>0.3±1.3</td>
<td>0.4±0.6</td>
<td>0.3±0.9</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>≥5</td>
<td>12±6.0</td>
<td>8.6±0.9</td>
<td>8.6±0.9</td>
<td>12±6.3</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>≤15</td>
<td>13±5.9</td>
<td>9.3±7.0</td>
<td>9.3±7.0</td>
<td>13±5.9</td>
</tr>
<tr>
<td>Plasma cells (%)</td>
<td>≤5</td>
<td>54</td>
<td>8.0±2.7</td>
<td>8.3±1.2</td>
<td>6.6±1.3</td>
</tr>
</tbody>
</table>

Figure 1.

Summary/Conclusions: As far as we know, this is the largest series of HMS bone marrow analyzed. Identification of common cytological findings in all the groups studied allows defining a characteristic cytological pattern for HMS. The reason for these findings could be related to an aberrant chronic immune response caused by a continuous exposure to malaria parasites. Only bone marrows of HIV infected patients presented additional specific alterations (decreased cellularity and high proportion of atypical lymphocytes). Some authors hypothesize that HMS could eventually evolve to chronic lymphocytic leukemia, hairy cell leukemia or splenic lymphoma with villous lymphocytes, so a special follow-up would be advisable for those patients with a high proportion of atypical lymphocytes.

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 hematological 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 total score of 1:4; acute appendicitis very unlikely; Score 5-7: acute appendicitis probable; Score 8-10: acute appendicitis definite. #<; negative; #: positive.

Summary/Conclusions: Acute appendicitis occurring during the neutropenic phase in HSCT patients could be diagnosed by the 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 empiric antibiotic regimen is usually based on the local epidemiology of the microorganisms and their antibiotic susceptibility profile. Antimicrobial guidelines for the management of sepsis in cancer patients in East Sussex Healthcare Trust (ESHT) recommend piperacillin/tazobactam as monotherapy and gentamicin is added in case of septic shock. Vancomycin is also added as a first line therapy if there is a suspicion of central line sepsis. Alternative therapies are ceftazidime or meropenem plus aminglycoside.

Aims: We intend to review the aetiology of BSI and check the effectiveness of the antibiotics used in ESHT in cancer patients.

Methods: This retrospective study was conducted at ESHT from January 2006 to December 2015. Demographic and laboratory data were collected from the Pathology information system.

Results: A total of 640 episodes of BSI occurred in 297 patients (159 male). Of the 297 patients, 239 had haematology malignancies while 54 had solid organ tumour. Four patients had both. The neutrophil count was <1 cells/10⁹ in 383 episodes and majority of BSI occurred in this group. A total of 802 organisms (477 and 325 organisms from neutropenic and non-neutropenic respectively) were isolated. Of 802, 406 Gram positive and 386 Gram negative organisms were isolated. Seven Mycobacterium species and three Candida species were isolated. Most common organisms in neutropenic patients were Coagulase negative Staphylococcus (CoNS) (22%), Klebsiella species (14%), Escherichia coli (13%), Streptococcus species (10%), Pseudomonas species (10%), Enterococcus species (8%) and Staphylococcus aureus (4%). In non-neutropenic patients, CoNS (29%), Escherichia coli (11%), Pseudomonas species (8%), Streptococcus species (7%), and Klebsiella species (5%) were isolated. Twelve Glycopeptide resistant Enterococci were isolated. Four Methicillin resistant Staphylococcus aureus were isolated. In addition, 15 Extended Spectrum Beta-lactamase producing Gram negative bacilli were isolated. Among Gram negative organisms, more than 91% isolates were sensitive to piperacillin/tazobactam, cefazidime and ciprofloxacin and higher sensitivity rates were reported in gentamicin and meropenem, Table1 summarises the effectiveness of antibiotics used.

Summary/Conclusions: This study shows an on-going trend towards Gram positive organisms causing BSI in cancer patients. The antimicrobial regimens used in ESHT are highly effective against commonly isolated organisms. An early diagnosis and timely administration of appropriate antibiotics are imperative in managing BSI. The identification and the antimicrobial susceptibility of the microorganisms causing BSI in cancer patients remain important to develop antimicrobial treatment strategies, and to prevent the spread of antimicrobial resistance.
Table 1. The sensitivity of antibiotic regimens used.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Sensitivity</th>
<th>Neutropenic patients</th>
<th>Non-neutropenic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole/linezolid plus parenteral</td>
<td>99%</td>
<td>100%</td>
<td>99%</td>
</tr>
<tr>
<td>Micafungin</td>
<td>99%</td>
<td>100%</td>
<td>99%</td>
</tr>
<tr>
<td>Caspofungin plus parenteral</td>
<td>99%</td>
<td>100%</td>
<td>99%</td>
</tr>
<tr>
<td>Liposomal amphotericin</td>
<td>97%</td>
<td>99%</td>
<td>97%</td>
</tr>
</tbody>
</table>

**PB1899**

**CHANGING TREND IN LOCAL BACTERIAL EPIDEMIOLOGY: EXPERIENCE IN ACUTE LEUKAEMIA PATIENTS HOSPITALIZED IN SINGLE HEMOTOLOGY UNIT**

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**Background:** The intense chemotherapeutic regimens and hypomellitotic agents to treat acute leukemia induce prolonged neutropenia with high risk of infections.

**Aims:** To analyze local microbial epidemiology we studied patients admitted to our ward.

**Methods:** All 100 cases of Acute Leukemia (AL) admitted in our ward from August 2013 to February 2017 received prophylactic antibacterial therapy with fluoroquinolones and were analyzed for weekly routine tissue culture screening and serial blood culture for fever. Six patients were Lymphoid AL and 94 were Myeloid AL. 41 patients were not eligible for intensive chemotherapy (for age and comorbidities) and were treated with hypomellitotic agents, while 59 were younger than 65 years and were treated with induction/consolidation chemotherapy three to seven regimes. 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 hypomellitotic treatment in particular decitabine (5 patients 18%). During 2014 we had 3 mortal septic shock for multiresistant gram-klebsiella and Pseudomonas. Since then we adopted in our ward, isolation of patients with gram negative (klebsiella or pseudomonas) to include culture positive, hygienic and sanitary practices with closing room for 48 hours and hand disinfection before entering and after leaving any patient rooms. We noticed a change of bacterial infections incidence in these 3 years in our ward: reduction klebsiella/pseudomonas multiresistant infections and emergency of E.coli and Staphylococcus septic shock not multiresistant.

**Summary/Conclusions:** More epidemiological analysis in several haematological ward are necessary to understand if it is a changing local microbial epidemiology or is the different management of neutropenic patients with acute leukemia and/or a different antimicrobial strategy to determine this changing trend.

**PB1900**

**UK SINGLE-CENTRE SERVICE EVALUATION TO DESCRIBE THE IMPACT ON HEALTHCARE RESOURCE USE OF LOCAL ANTIFUNGAL PROPHYLAXIS AND TREATMENT PROTOCOLS IN THE MANAGEMENT OF HIGH-RISK PATIENTS WITH NEUTROPENIA**

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**Background:** Patients with neutropenia, including those with haematologic malignancies, are at high risk of invasive fungal infections (IFI). Pre-2014, there were no formal written guidelines but the guidance at Poole Hospital NHS Foundation Trust specified the use of posaconazole oral suspension for primary prophylaxis in all high-risk patients except those with acute lymphoblastic leukaemia (ALL). In 2014 formal guideline changes included the introduction of the tablet formulation of posaconazole, use of micafungin as first line empirical therapy and a focus towards improving diagnostics to guide management. MSD Ltd. has developed the Fungal Service Evaluation Tool (FSET), a secure database and analysis tool, to support UK clinicians managing patients at risk of breakthrough IFI (BIFI) to evaluate their antifungal management.

**Aims:** This service evaluation aimed to utilise the FSET to evaluate the impact of the antifungal management guidelines on healthcare resource utilisation associated with patients at risk of a BIFI.

**Methods:** An interim analysis of high-risk adult patients with prolonged neutropenia aged ≥ 18 years at initiation of antifungal prophylaxis/treatment was carried out. Retrospective data on patient characteristics, antifungal prophylaxis and treatment, IFI-related diagnostic tests, hospital attendance/admission during antifungal prophylaxis were collected for 12-month periods before and after 2014 (Cohort 1: 2013; Cohort 2: 2015). Anonymised data was entered into the FSET and this data was analysed using descriptive statistics.

**Results:** The evaluation included 24 patients in Cohort 1 (median age 66.8 [interquartile range (IQR): 47.5–72.2] years; 16 [67%] male; 5 [21%] ALL) and 22 patients in Cohort 2 (median age 66.8 [IQR: 51.7–73.4] years; 13 [59%] male; 1 [5%] ALL). At least one line of antifungal prophylaxis was recorded in 22 (92%) patients in Cohort 1 and 17 (71%) in Cohort 2. Posaconazole was the most commonly prescribed antifungal in Cohort 1 (18/24 [75%]) and Cohort 2 (17/22 [71%]). Other agents used included liposomal amphotericin B, fluconazole, and itraconazole. There were no patients in Cohort 1 and 2 (9%) patients in Cohort 2 (overall 4%) who experienced a BIFI: 1 was defined as confirmed and 1 as suspected. The mean 12 month costs per patient for all resource utilisation (including antifungal drug costs, hospitalisation costs [including admissions and attendances], investigations and tests) was £28,903 in Cohort 1 and £21,934 in Cohort 2 (Figure 1). Hospitalisation costs were a key determinant of overall costs, which is common in the management of people with complex underlying disease. There were 4 (17%) patients in Cohort 1 and 1 (5%) in Cohort 2 who had a period of ITU associated stay, which typically has greater costs than general wards. The most common investigations/tests were blood cultures (Cohort 1: mean 13.8; Cohort 2: mean 10.7) and chest x-ray (Cohort 1: mean 4.0; Cohort 2: mean 2.5), which are in line with routine clinical practice. Once implemented, the guideline was adhered to in the management of 19 patients (86%) in Cohort 2.

**Figure 1. Breakdown of mean 12 month resource utilisation costs for cohorts 1 and 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.
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, or high serum ferritin, or high dropout rate of approximately 50% of patients within one year). 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 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 unsuitability and of treatment discontinuation were extracted from our database. 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. Results: A total of 476 otherwise healthy children were analyzed. Mean SpHb value was 11.9 ± 9.3 g/dl (range 9-16 g/dl). Overall, the prevalence of anemia in the entire population was 34.5%. Anemia was present in 5/31 (16.1%) of the children within group 1, 9/78 (35.9%) in group 2, and 54/81 (40%) in group 3. (p=0.039, two sided Pearson’s Chi square). There were no differences in the prevalence of anemia by gender in groups 1 and 2. In group 3, anemia was significantly more prevalent in females 32/65 (49.2%) than in males 22/48 (31.4%), p=0.035. Aims: To detect iron deficiency anemia early and to reduce the adverse impact by IDA, we assessed 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.

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: 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 for 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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1Servio de Hematologia Clinica, Centro Hospitalar e Universitario de Coimbra, Coimbra, Portugal

Background: Sideropenic anemia (IDA) is the main cause of anemia worldwide. Even though, its diagnosis is quite straightforward with the use of red blood cell indices, peripheral blood smear (PBS) and ferritin measurements, there are still some pitfalls, namely in the presence of inflammation. The chelation of iron by protoporphyrin constitutes the final reaction of heme biosynthesis. In the absence of iron, zinc becomes an alternative substrate for ferrochelatase leading to the formation of zinc protoporphyrin (ZPP). This compound can be quantified by fluorometry in blood samples, proving itself as a useful and easy parameter for the diagnosis of IDA. However, this technique is not broadly used in the clinical practice.

Aims: Determine the cut-off value of ZPP for the diagnosis of IDA. Evaluate the value of ZPP for the differential diagnosis between IDA and anemia due to inflammatory diseases (AID).

Methods: We have analyzed in our lab, from 1st to 15th February 2015, all the complete blood samples (pediatric and adult) with anemia (as defined by WHO) which had sedimentation rate (SR) and serum ferritin evaluations.

We have defined three different groups: IDA: Anemia and Ferritin <20μg/L; AID: Anemia, Ferritin >20μg/L and SR>20mm/h; Control group (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, Biomedicala, 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 statistically significant a p-value <0.05.

Results: We have identified 204 samples that fulfilled the inclusion criteria: 104 with IDA, 51 with AID and 49 from control patients. IDA group: 73% female (F); mean age 32.3y 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[SDD246.2] and ZPP 136.7 μmol [SD 107.8]; mean SR 47mm/h [SD 21]; GC: 69.4% F; mean age 44.8y in F [1-79], and 37y in M [2-65 years]; mean Hb 13.8 g/dl [SD 0.9]; mean ferritin 71.9ug/L [SD 49.9] and ZPP 78.6 μmol [SD 26.6]; 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 our study ZPP was not a reliable method to differentiate IDA from AID. This could be due to a sample selection bias (since clinical data were missing and the number of patient with AID was substantially lower than with IDA). It would be important to enlarge the AID sample in order to obtain a more reliable result. Since ZPP measurement can be performed in capillary blood and it is a very quick and cheap method to diagnose IDA, this could be a powerful tool in under-developed countries.

PB1905

HYPERFERRITINEMIA AND SERUM INFLAMMATORY CYTOKINES IN ADULTS WITH NEWLY DIAGNOSED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS ASSOCIATED WITH HEMATOLOGICAL MALIGNANCY

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Background: Hemophagocytic lymphohistiocytosis (HLH) is an underdiagnosed but life-threatening syndrome of hyperinflammation which in adults is often caused by hematological malignancies. Release of inflammatory cytokines in this HLH induces the lymphocytes and cells and cytokine production that cumulates in cytokine storm and hyperinflammation. Hyperferritinemia ≥500 μg/L is a diagnostic criterion for HLH. Prevalence of hyperferritinemia in HLH in the adult population is much less established than in children.

Aims: The aim of the present study was to evaluate the frequency and extent of hyperferritinemia as well as serum concentrations of selected inflammatory cytokines at the time of diagnosis of hematological malignancy-associated HLH (HM-HLH) in adults.

Methods: The study included 71 adults with HM-HLH, aged 22–84 years, and diagnosed between 2009 and 2016. Hematological malignancy was defined as a neoplasm of lymphoid origin (≥1% blasts) in bone marrow. In all studied patients, the diagnosis of HLH was based on the HLH-2004 criteria. Since the majority of patients in this study had severe lymphophenia, we decided to not perform functional analyses of NK-cells for HLH diagnosis. Thus, we included in this analysis all patients with hematological malignancies and suspected HLH who fulfilled at least four of seven HLH-2004 criteria as well as at least two of three additional features: sIL-2Ra ≥2400 U/mL, hemophagocytosis in BM, and hyperferritinemia ≥10,000 μg/L. Serum concentrations of inflammatory cytokines IL-1β, IL-6, IL-8, IL-10 and TNF-α were analyzed using chemiluminescence (IMMULITE® system, DPC Siemens). Serum levels of sIL-2Rα were determined by ELISA, using the quantitative ‘sandwich’ enzyme immunoassay, on the IMMULITE® 1000 Immunoassay System (DPC Siemens).

Results: Lymphoid malignancy was diagnosed in 42 patients and myeloid malignancy in 29 patients. Fifty-four (76%) patients developed HLH as a first manifestation of an unknown malignancy, during progressive disease, or at malignancy relapse. The remaining 24% died before HLH chemotherapy. Serum ferritin concentration (ref.: 30–350 μg/L) at the time of HM-HLH diagnosis was elevated in all but one patient (70/71, 97.9%). Mean ferritin was 37.9±84.44 μg/L, median value 14.72 μg/L, and ferritennia range 96–645.29 μg/L. As HLH-2004 criteria, hyperferritinemia ≥500 μg/L was present in 69 of 71 patients (97%) at the time of HLH diagnosis. Hyperferritinemia of ≥2000 μg/L was noted in 67 (94%) patients, hyperferritinemia of ≥5000 μg/L in 56 (79%) patients, and hyperferritinemia of ≥10,000 μg/L occurred in 42 (59%) patients. Serum levels of sIL-2Rα (sCD25) were measured in 69/71 patients, among whom 91% (63/69) had values ≥2400 U/mL. Moreover, in 54 patients sIL-2Ra was clearly elevated to 2129, 2233, and 2345 U/mL, respectively. Concentrations of TNF-α, IL-6, and IL-10 in serum were in each elevated in over 85% of the examined hM-HLH patients. IL-8 concentration was increased in half of all tested patients at the time of HLH diagnosis. However, IL-1B concentration was above reference range only in 12% of patients (7 of 58). Results of the inflammatory cytokine analyses in patients with newly diagnosed hM-HLH are presented in Table 1.

Table 1. Inflammatory cytokines in patients with newly diagnosed hM-HLH.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Mean (μg/L)</th>
<th>Median (μg/L)</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>0.06 (0.03)</td>
<td>0.06 ± 0.03</td>
<td></td>
<td>(0.01, 0.15)</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.86 (0.49)</td>
<td>0.86 ± 0.49</td>
<td></td>
<td>(0.45, 1.68)</td>
</tr>
<tr>
<td>IL-8</td>
<td>0.95 (0.48)</td>
<td>0.95 ± 0.48</td>
<td></td>
<td>(0.50, 1.60)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>26.7 (18.3)</td>
<td>26.7 ± 18.3</td>
<td></td>
<td>(4.8, 60.2)</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Hyperferritinemia at the time of HLH diagnosis was common in Swedish adult patients with HM-HLH. Hyperferritinemia ≥500 μg/L was present in vast majority (97%) of them. We would like to emphasize that serum ferritin level fluctuations and can differ significantly from one day to another. Ferritinemia should be repeatedly measured in cases of suspected HLH. Serum concentrations of TNF-α, IL-6, IL-8, and IL-10 were frequently elevated in the examined hM-HLH patients and these can become important markers supporting HLH diagnosis in equivocal cases. On the other hand, IL-1β seems to be less useful in confirming a cytokine storm in this patient group.
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) are considered as markers of endothelial dysfunction. Asymmetric dimetil arginine (ADMA) is known as another marker of endothelal dysfunction in this study: we aimed to evaluate circulating EMPs and ADMA in children with iron deficiency and iron deficiency anemia and to disclose iron deficiency with the strongest relation with EMPs, ADMA and carotid atherosclerosis.

**Methods:** This study included 30 children with iron deficiency anemia, 30 children with iron deficiency without anemia and 30 healthy children whose anthropometrics measurements were recorded. Hemoglobin, serum iron level, iron binding capacity, ferritin, and lipid profile were studied. Circulating EMPs (CD144, CD146, and CD105) were measured by flow cytometry. ADMA was measured by ELISA. The carotid artery intima media thickness (CIMT) and left ventricular mass index (LVMI) were measured using echocardiography.

**Results:** CD144 and CD105 EMP levels were lower in the iron deficiency without anemia group than in the control group and statically lower than in the iron deficiency anemia group (p<0.05). There were no significant differences in ADMA level between groups. Any significant variant in ADMA level was not observed between groups. CIMT was negative correlated with ferritin and high density lipoprotein and positive correlated with body weight.

**Summary/Conclusions:** In this study, endothelial dysfunction which occurs as a result of iron deficiency were observed. According to our result, CD144 and CD105 EMP levels in the iron deficiency without anemia group were lower than the iron deficiency anemia and control group; these levels in iron deficiency anemia group were higher than control group. In addition, when the level of ferritin has decreased, CIMT has increased. This study show that CD144 and CD105 may be related to endothelial dysfunction which occurs by iron deficiency.

**PB1908**

**INVESTIGATION OF IRON METABOLISM FOR REGULATING MEGAKARYOPOIESIS AND PLATELET COUNT ACCORDING TO THE MECHANISMS OF ANEMIA**

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**Background:** Iron deficiency anemia (IDA) is characterized by depletion of total body iron stores. By contrast, chronic inflammation makes iron unavailable for hematopoiesis through a cytokine-mediated cascade, resulting in anemia of chronic inflammation (ACI). 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 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.
ROLE OF PRO-PHAGOCYTIC CALRECTICULIN AND ANTI-PHAGOCYTIC CD47 IN MDS AND MPN MODELS TREATED WITH AZACITIDINE OR RUXOLITINIB

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Background: Myelodysplastic syndrome (MDS) and Myeloproliferative neo-plasms (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-doubling 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 6-8 fold increase in CD47 increase 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 5-8 fold increase in CD47, which would be expected as RUXO is used to treat MPN in humans. When the MDS/MPN cell model or pure MDS models were treated with RUXO, the ratio of CALR/CD47 decreased substantially (with CALR expression only increased 2.4-3.7 fold compared to CD47 increasing 4.6-6.9 fold) showing resistance to treatment and a significant anti-phagocytic response. Interestingly one of the MDS cell line models (MOLM-13) showed an unexpectedly good response to RUXO therapy with high CALR/CD47 ratio (8 fold vs 4.8 fold, respectively).

Summary/Conclusions: In line with results in solid tumours, we have shown that treatment for MDS and MPN leads to an up-regulation of CALR and, to a lesser extent, CD47 in cell line models. The ratio of CALR/CD47 seems to correlate with specific treatment response, significantly increasing when given diseases models are treated with the appropriate drug. We postulate a role of CALR expression in leukaemia cell phagocytosis, with CD47 co-expression in synergy as a protective instinct within the cell to try and prevent apoptosis. Some solid tumour models showed excessive rise in CD47 expression and low expression of CALR. This indicates that the CD47 mediated anti-phagocytosis takes control and suppresses the CALR expression, leading to cancer cell survival and ineffectiveness of treatment. These results need to be validated in human samples at different stages of disease to allow a better understanding of treatment response and/or resistance to chemotherapy within these diseases.

PB1910

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 erythroid differentiation 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 5q- MDS patients. Recently, the pivotal role of RPS14 in human erythropoiesis during 5q- MDS pathology has been demonstrated: RPS14 haploinsufficiency produces the activation of p53 and its target p21 in erythroid cells, resulting in cell cycle arrest and apoptosis. Based on these results, non-5q- patients expressing low levels of RPS14 will be potentially beneficed by lenalidomide therapy. In this work, we explore the origin of the altered RPS14 expression in non-5q- patients and its potential link with 5q-pathology.

Aims: The objective of this work was to explore the origin of RPS14 low expression in non-5q- MDS patients and its link with 5q-pathology. In order to do this, we analysed potential mutations in RPS14 gene. We also studied expression changes in other key genes involved in the development of the 5q- disease, including the tumour suppressor gene SPARC and the putative tumour suppressor gene CSNK1A1, contained in the commonly deleted region. Moreover, other 32 genes related with MDS disorders were evaluated in relation with RPS14 levels. Finally, in order to establish if this group of patients could be beneficed by lenalidomide therapy, p21 expression levels were also analysed.

Methods: DNA and RNA were extracted from the bone marrow of 89 non-5q- MDS patients. Ten controls and nine 5q- MDS patients were used as negative and positive controls, respectively. RPS14, SPARC, CSNK1A1 and p21 mRNA levels were analysed by real time PCR using Taqman probes and a 7500 RT-PCR System. β-glucuronidase gene was used as endogenous reference to normalize data. Samples were classified by RPS14 expression levels and differences in SPARC, CSNK1A1 and p21 expression mean values between the two groups were analysed using the Mann-Whitney U test. RPS14 and 32 genes expression were also analysed using Ion Proton system.

Results: Non-5q- patients expressing low levels of RPS14 presented higher survival probability in the IPSS lower risk group. This data, in addition with a tendency for increased p21 expression, suggests that this group could be beneficed by lenalidomide therapy. Nevertheless, we did not observe a significant correlation between RPS14 expression and the degree of dysplasia in non-5q- MDS patients. Moreover, we found no association between RPS14 expression and the degree of dysplasia in non-5q- MDS patients. In addition, the majority of patients analysed did not present any mutation in RPS14 gene. Only two MDS patients showed mutations upstream, downstream or within intronic regions of the gene. The origin of these mutations remain unknown.

Summary/Conclusions: Although the importance 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 counts (CBC), the fastest laboratory investigation, performed on the recently developed XN-10®, including classical parameters (hemoglobin level, Mean Corpuscular Volume (MCV), reticulocytes, plateletes, neutrophils and extra-parameters i.e. plateletes by fluorescense (PLT-F), immature plateletes fraction (IPF%), immature reticulocytes fraction (IRF%) and the neutrophils median position. Of the three axes as well as their dispersion (Neut-WX). For comparison with normal values, results from 707 healthy subjects over 50 years old, for whom CBC were performed on the same analyzer and generated no flag, were used. All had parameters within the normal range according to age. According to the WHO, 37 participants were classified in the MDS-EB, 35 in MDS with single lineage dysplasia (MDS-SLD) and MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD) within the MDS group and by comparison with controls as described by the WHO 2016 classification.

Methods: One hundred and thirteen patients were enrolled in the study, for whom a diagnosis of MDS was concluded based on CBC, bone marrow smears examination and karyotype. All patients were free of treatment, including transductions, at inclusion. They were 63 men and 50 women with a median age of 82 years (range 36-96 yo). CBC were performed on a Sysmex analyzer XN-10®, including classical parameters (hemoglobin level, Mean Corpuscular Volume (MCV), reticulocytes, plateletes, neutrophils and extra-parameters i.e. plateletes by fluorescense (PLT-F), immature plateletes fraction (IPF%), immature reticulocytes fraction (IRF%) and the neutrophils median position. Of the three axes as well as their dispersion (Neut-WX). For comparison with normal values, results from 707 healthy subjects over 50 years old, for whom CBC were performed on the same analyzer and generated no flag, were used. All had parameters within the normal range according to age. According to the WHO, 37 participants were classified in the MDS-EB, 35 in MDS with single lineage dysplasia (MDS-SLD) and MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD) within the MDS group and by comparison with controls as described by the WHO 2016 classification.

Aims: The primary endpoint was to discriminate MDS patients from normal samples and the secondary end-point was to distinguish MDS with excess blasts (MDS-EB), MDS with multilineage dysplasia (MDS-MLD), MDS with single lineage dysplasia (MDS-SLD) and MDS with ring sideroblasts and single lineage dysplasia (MDS-RS-SLD) within the MDS group and by comparison with controls as described by the WHO 2016 classification.

Results: Both classical and extra parameters indeed showed significant differences between the subgroups tested. Among the whole group of MDS patients a number of parameters of all lineages were statistically different from the healthy cohort. The median level of hemoglobin was 9.92±1.96 g/dL (p<0.0001), the median MCV (291,24±10.56 fl (p<0.0001), reticulocyte count 44.3±10.7% (range 8-120, p=0.016, 16.7% (range 0.2-14.0); p<0.0001). The neutrophil count was 154±51x10⁹/L (p<0.0001) and the median IPF% 12.4% (2.5-20.5; p<0.0001). The median platelet count was 194±128x10⁹/L (p<0.0001), and the median IRF% 8.8% (1.2-8-165.9; p=0.041) and IRF% 16.7% (range 2.4-50.9; p<0.0001). The median erythropoietin levels and greater expression of CD34 / CD117 / IL-6 / TNFa. Assigning a value (+1) to each altered variable a new prognostic score was obtained, which we named Progression Score for Acute Leukemia. We observed that patients belonging to subtypes with the highest scores were those that progressed to AML, namely RAEB-1, RAEB-2 and 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 etiology. 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 syndromes (MDS) and acute myelogenous leukaemia (AML) patients.

Aims: To clarify PU.1 molecular function we investigated the gene expression of PU.1 and JDP2 in MDS, AML and normal controls. As well as providing a marker for the development of new therapeutic strategies to manage MDS, we also aimed to investigate the gene expression of PU.1 and JDP2 in MDS, AML and 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 2⁰ 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 JDP2 expression <R=0.9333, s=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 MDS using shRNA. Interestingly, the shRNA 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 in treatment response.

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 β-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). screening by R-banding karyotype and metaphasic and interphasic FISH using a panel including six probes (5q-, 7q-, 20q-, del(17p13), MLL, Inv(3) t(3;3).

**Results:** Among these 101 pts, 58 were male with a sex ratio=1, 35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin of patients was 80 g/L (29-150), more than 60% of patients had severe anaemia mainly in the low risk group; the median absolute neutrophil count was 3 G/L (0.060-13, 5), and the median platelet count was 144 G/L (5-659). Median bone marrow blast value was 4% (0-18). Cases were classed by cytology morphology FAB as RA (n=45), REAB (n=34), RARS (n=16), other (n=6). Classification by WHO 2008 included CRDU (n= 31 of which RA : 18, RT : 10, RN : 3), CRDM (n= 16), RAEB-1 (n=22), RAEB-2 (n=13), RARS (n=15), Isolated 5q- (n=4). Among 101 patients, cytogenetic abnormalities by R banding karyotype (n= 84) and FISH (n=101) were found in 41 cases (41%) distributed as single anomy (n= 19), double anomaly (n=5) and complex (n=17). The main cytogenetic abnormalities seen were isolated 5q deletion (n=4), isolated 7q deletion (n=2), isolated 20q deletion (n= 6), isolated trisomy 8 (n=2), 17p13 deletion (n=6), -Y (n=1), complex aberrations ≥3 (n=6), complex aberrations ≥5 (n=6), complex aberrations ≥7 (n=5), others (n=3). IPSS was assessed in 84 patients: 27% (low risk), 44% (intermediate 1), 24% (intermediate 2), 5% (high risk). IPSS-R was assessed in 84 patients (18% very low risk, 30% low risk, 22.5% intermediate, 15.5% High risk, 14% very High risk). Leukemic transformation into AML occurred in 33% of patients in a median time of 12 months. According to IPSS, the median OS time survival is not reached for low risk group, 41 months (m) for Intermediate 1 risk, 11 m for Intermediate 2 risk, and 4 m for High risk. According to IPSS-R, the median OS time survival is not reached for Very low risk, 43 m for low risk, 24 m for Intermediate risk, 18 m for High risk and 4 m for very high risk.

**Summary/Conclusions:** Our results are in agreement with those previously published regarding demographic features, distribution of recurring cytogenetic abnormalities and prediction of survival. Myelodysplasias are among the most difficult haematological diseases to treat. Treatment of low risk and high risk myelodysplasia are completely different, the last group carrying a great risk of leukemic transformation. For all these reasons, application of the new tools to classify MDS is of an major importance. This is especially true in emerging countries where few therapeutic means are available, hence the need to predict the prognosis of these diseases in order to better target treatments. To the best of our knowledge, it is the first study conducted in our country.
Myelodysplastic syndromes - Clinical

PB1918

CLINICAL EVOLUTION OF ACUTE MYELOID LEUKEMIA WITH MYELODYSPLASIA-RELATED CHANGES
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Background: Acute myeloid leukemia (AML) with myelodysplasia-related changes (MRC) is usually classified associated to worse clinical course and poor prognosis compared other AML subtypes. Differences between treatment modalities according to age, and the response to treatment, would help to provide specific anti-AML treatment in this difficult scenario.

Aims: The objective of this study is analyze the clinical features and course of patients with AML with MRC, in order to evaluate the impact of different therapeutic regimens in this subgroup.

Methods: We report an unicentric retrospective study of 76 patients with AML with MRC, over the past ten years in a single institution in Spain. We analyzed the overall survival (OS) among the subgroup of patients with over or under 65 years, and the different types of treatment that has been offered.

Results: Median age was 69 years with a male predominance, and 66% was preceded by a known myelodysplastic syndrome with a median interval of 18 months to progress to AML. The more frequent genetic abnormalities in descending order were trisomies, del(5q), and del(7q)/-7. The patients aged >65 years had <65 were 70% and 30%, respectively. The patients aged >65 received DNA hypomethylating agents (40%), anthracycline-cytarabine combinations (9%), low-dose cytarabine or hydroxyurea (17%), and supportive measures (34%). The patients aged <65 received induction chemotherapy with anthracycline-cytarabine combinations so as to continue with post-consolidation management with allogeneic transplantation, but the 44% died over the induction chemotherapy (OS: 2.2 months). The OS in patients aged >65 was 20.2 months in chemotherapy plus allogeneic transplantation. The OS in patients aged >65 was 2-3 months in the group of anthracycline-cytarabine combinations, 3.81 months in the DNA hypomethylating agents group, 2.8 months in the low-dose of AraC/hydroxyurea, and 0.5 months in supportive measures group (Figure 1).

Figure 1.

Summary/Conclusions: The AML with MRC patients is a group with difficult treatment decisions and poor prognosis, in whom only the chemotherapy plus allogeneic transplantation treatment manage long-term survival. In patients aged >65, there is not a significant difference among groups, although the chemotherapy with anthracycline-cytarabine seems to reach a better OS versus other available treatments.

PB1919

SAFETY, EFFICACY, AND PHARMACOKINETICS OF INTRAVENOUS RIGOSERTIB IN JAPANESE PATIENTS WITH RECURRENT/RELAPSED OR REFRACTORY MYELODYSPLASTIC SYNDROMES: A MULTICENTER, OPEN-LABEL, PHASE I STUDY
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Background: Rigosertib, a novel phosphoinositide 3-kinase inhibition, selectively induces the apoptosis of cancer cells and is safe and well tolerated in pts with recurrent/relapsed or refractory MDS.

Aims: We conducted a multicenter, open-label, Phase I study of intravenous rigosertib to evaluate its safety, efficacy, and pharmacokinetics and to determine the recommended dose (RD) for Japanese pts.

Methods: The key eligibility criteria were as follows: recurrent/relapsed or refractory MDS; age: 20 or older; FAB classification (RA, RARS, RAEB, RAEB-t, and CMML), excepting patients at IPSS low- or Int-1 risk with respect to RA; ECOG PS of 0 to 2; no major organ dysfunction; and written informed consent. Treatment was administered intravenously, 1,200 mg/day 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, RAEB-t, and RA, respectively. There were 5, 2, and 1 pts in the 1,200, 1,500, and 1,800 mg arms, respectively. The median numbers of delivered cycles in the 1,200 and 1,800 mg arms were 4 (2 to 4) and 2 (1 to 8), respectively. DLT occurred not in the 1,200 mg arm but in the 1,800 mg arm: 5 episodes of grade 3 non-hematological toxicity in 2 pts. One pt developed 3 cases of sepsis and meningitis, and the other 3 episodes of hypochloremia, 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 thrombocytopenia, grade 4 neutropenia, and grade 3/4 leukopenia, and grade 3/4 leukopenia, as well as 1 case each of grade 3 lymphopenia were observed. In the 1,800 mg arm, 3 cases of grade 3/4 neutropenia, 2 cases each of grade 3 lymphopenia, and grade 4 thrombocytopenia, and grade 3/4 neutropenia, as well as 1 case each of grade 3 lymphopenia, increased C-reactive protein, erythropenia, and hypochloremia 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 participating in a global randomized Phase III study to compare rigosertib with physicians’ choice of treatment.

PB1920

IRON CHELATION THERAPY IMPROVES HAEMATOLOGICAL RESPONSE IN HIGH-RISK MYELODYSPLASTIC PATIENTS TREATED WITH AZACITIDINE
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Background: The goals of treating older patients with Myelodysplastic Syndrome (MDS) are different than for younger patients. Few elderly patients are able to pursue an allogeneic stem cell transplant. Azacitidine (AZA) improves hematologic remission, hematological improvement, and cytogenetic response in high-risk MDS patients treated with 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. Anemia 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 anemias. Its efficacy has been established in controlled clinical trials.

Aims: We report our experience on using the azacitidine in patients with high-risk MDS, evaluating the efficacy and safety. Concomitant treatment with deferasirox was performed in a routine clinical setting following Consensus Guidelines on Iron Chelation Therapy.

Methods: In our Institution from October 2009 to January 2017 we have...
treated 32 elderly patients (19 male and 13 female, median age 76 years, r. 71-88) affected by HIGH-RISK MDS (IPSS INT-2/HIGH). Patients received subcutaneous azacitidine at 75mg/m2 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 1-6). The 4-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? I. Koutsavlis1,*, J. Falconer2, J. Fleming3, H. Roddie1

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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, haemolysis, RBC transfusion reactions and potentially severe clinical consequences for the transfused patient. Collectively, alloimmunization appears to be higher in patients with myelodysplasia (MDS) and chronic myelomonocytic leukaemia (CMML) with a rate somewhat around 15%. Identification of patients at risk of developing alloantibodies would be of clinical significance as antigen negative red cells could be crossmatched in advance for use in clinical practice. 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 cytotogenic 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 available in 228 of the above patients and uni- and multivariate analysis was performed separately.

Results: The mean age at diagnosis was 73 years (range 20-95) with 58.3% male patients. Overall, 45 patients (12.5%) formed 76 antibodies [88 alloantibodies, 42 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), Cw1/Jka1/Kp (5 cases each), Lua (4), e/Fya (3 cases each), M (2), c/D’/c’/D’/c’/Bga (1 case each). Collectively, alloantibodies against the Rh and Kell systems encountered in patients with MDS: an analysis of the erythropoietic response to ESAs therapy in a cohort of anemic non transfusion-dependent MDS patients, enrolled in a retrospective register, RECAMDS, subgroup of Italian register, was performed.

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, cytogenetic profile) reached statistical significant level as predictors for immunization; age (p=0.59), sex (p=0.07), MDS WHO subtype (p=1.0). 228 patients had known cytogenetics at diagnosis, 86 patients (37.8%) were low risk, 32 patients (14%) were intermediate-II risk group, and 9 patients (4.0%) were high risk group. The incidence of acute and chronic GVHD was 51.1% and 28.6% respectively. The 5-year estimate for overall survival of the whole group was 21.8%. In univariate analysis, covariates associated with a better OS were recipient age <40 years (p=0.02) and the presence of cGVHD (p=0.002). On multivariate analysis the presence of cGVHD is significant predictor of survival (p=0.04). Also cGVHD significantly improve the OS for low and high risk MDS group (p = 0.02 and 0.03 respectively). While presence of acute GVHD, IPSS & interval between diagnosis and transplant were not significantly affect OS (p>0.05). The 5-year estimate for DFS of the whole group was 28.6%. On multivariate analysis the presence of cGVHD significantly reduce relapse (p=0.029).

Summary/Conclusions: The presence of cGVHD significantly improved OS and reduced the risk of relapse in patients with MDS. We also found that the presence of cGVHD significantly improved OS especially in high-risk patients group, which suggests that the GVLF effect may be beneficial in high-risk patients who do not receive intensive preparative regimens.

PB1922

PROGNOSTIC MARKERS THAT PREDICT THE OUTCOME OF REDUCED INTENSITY CONDITIONING TRANSPLANT IN ADULT PATIENTS WITH MEYLODYSPLASTIC SYNDROMES: A SIN clinico-EXPERIENCE S. Eilashwah1,*, S. Shamaa2, H. Kamel3, M. Samra4, E. Azmy1

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Background: Myelodysplastic syndromes (MDS) are a heterogeneous group of hematologic diseases, characterized by a clonal abnormality of hematopoietic stem cells. The incidence of MDS is age-dependent. The treatment approach is to categorize patients into lower or higher risk MDS and to select a suitable treatment accordingly. HCT offers potentially curative therapy for patients with MDS. Lower intensity conditioning (RIC) regimen was used to reduce the toxicities associated with transplant procedure. The main concept of RIC relies upon adoptive immunotherapy especially in the low risk patients allowing the graft versus leukemia to occur.

Aims: This study aimed to investigate the occurrence of allogenetic peripheral blood stem cell transplantation and the intensity conditioning regimens 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 between 1980 and 1998. According to housing regimen, 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 was 51.1% and 28.6% respectively. The 5-year estimate for overall survival of the whole group was 21.8%. In univariate analysis, covariates associated with a better OS were recipient age <40 years (p=0.02) and the presence of cGVHD (p=0.002). On multivariate analysis the presence of cGVHD is significant predictor of survival (p=0.04). Also cGVHD significantly improve the OS for low and high risk MDS group (p = 0.02 and 0.03 respectively). While presence of acute GVHD, IPSS & interval between diagnosis and transplant were not significantly affect OS (p>0.05). The 5-year estimate for DFS of the whole group was 28.6%. On multivariate analysis the presence of cGVHD significantly reduce relapse (p=0.029).

Summary/Conclusions: The presence of cGVHD significantly improved OS and reduced the risk of relapse in patients with MDS. We also found that the presence of cGVHD significantly improved OS especially in high-risk patients group, which suggests that the GVLF effect may be beneficial in high-risk patients who do not receive intensive preparative regimens.
TRANSFORMATION TO ACUTE LYMPHOBLASTIC LEUKAEMIA

CHARACTERIZATION OF MYELODYSPLASTIC SYNDROMES WITH TRANSFORMATION TO ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Myelodysplastic syndromes are heterogeneous diseases with variable probability of developing a transformation to acute leukaemia. The vast majority of these cases present a transformation to acute myeloid leukaemia. We here describe a series of 4 cases of MDS/CMMML with evolution to acute transformation into lymphoblastic leukaemia. These events are very rare and are to 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 one, 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) 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 number of MDS cases we observed a transformation to ALL. Further studies are needed to confirm this hypothesis, and molecular examination is needed to characterise the event enabling a myeloid phenotype to switch to a lymphoblastic one. The reason why del(5q) seems to be present in a high proportion of MDS patients transforming to ALL is not clear; further studies need to be performed.

VITAMIN D IS ASSOCIATED WITH SEVERITY OF DISEASE AS EXPRESSED BY SUBDIAGNOSIS AND IPSS-R IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND RELATED DISEASES

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Background: Recent findings indicate that vitamin D (VD) might impact hypomethylation therapy of myelodysplastic syndromes (MDS). Epigenetic activity of VD is mainly mediated through interaction with its nuclear receptor (VDR). Activated VDR binds to specific genomic sequences (VD response elements) which influence gene transcription by histone modification, mainly acetylation but also demethylation. Among genes affected by VD/VDR is BGLAP encoding for the non-collagenous protein osteocalcin (OCN) produced by osteoblasts and implicated in osteogenesis. Furthermore, it has been shown that OCN is expressed by activated hematopoietic stem cells in hematological malignancies.

Aims: We initiated an exploratory study, collecting patients’ data on serum VD, and osteocalcin (OCN)-levels in 59 unsellected patients with MDS, MDS/myeloproliferative neoplasm (MPN) and secondary acute myeloid leukaemia (sAML).

Methods: Serum VD levels were assessed by measuring 25-hydroxyvitamin D (25(OH)D), the biochemical indicator of VD status. Analysis was done by clinical chemistry. VD levels were classified as follows: <20 ng/ml <50% response, 20-30 ng/ml (50-75% response), 30-50 ng/ml (75-90% response), >50 ng/ml (90-100% response). Furthermore, immunohistological expression of OCN was investigated by immunohistochemistry. Immunohistological expression of OCN was evaluated by semi-quantitative analysis.

Results: We found median serum 25(OH)D levels (normal range 30-100 ng/ml) of 16 ng/ml (RA, RARS, n=35), 23 ng/ml (RAEB-1/2, n=15), and 20 ng/ml (MDS/MPN, n=8) (p=0.273).When classified by IPSS-R, median serum 25(OH)D levels were 18 ng/ml in “(very) low” (n=20), 16.5 ng/ml in

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 occurrence is a result of genetic alterations that differ in frequency of appearance, the duration of the course and the probability of transformation into acute leukemia. The choice of therapy for a particular patient is determined by the morphological variant of the disease, the prognostic group, age and comorbidity. In hypoplastic cases of MDS are often used immunosuppressive therapy.

Aims: Analysis of the effectiveness of immunosuppressive therapy in patients with primary MDS

Methods: The research included 19 patients with primary MDS from 22 to 58 years (median age 46 years, 11 male, 8 female). The diagnosis was made according to the criteria of the WHO classification of the WHO classification of the WHO 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%, RARS in 31.6, RAEB in 15.8. Hypoplastic forms of MDS were diagnosed in 63.2% patients. The increased number of lymphocytes in the bone marrow of patients 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 remis- sion (normalization of blood and bone marrow), partial remission (improvement of blood counts for more than 50% and no dependence on transfusions of blood components) or improvement (reduction in transfusion requirements by 50% or more). Complete remission was achieved in 10.5% of patients (only variant RA). Partial remission was obtained in 31.8% (variants RA and RCMD) and disease progression in 36.8% (variants RA, RAEB and RARS). There was no response to treatment in 21.1% of patients (variants RCMD and RAEB). Positive effect on immunosuppressive therapy significantly more likely achieved in patients with hypoplastic forms MDS (57.9%) and the presence of clusters of lymphocytes in the bone marrow biopsy (36.8%). Dependence of treatment efficiency and cytogenetic 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 immunosuppres- sive therapy can be expected in patients with hypoplastic MDS and accumulation of lymphocytes in the bone marrow biopsy.
Results: strategies of JMML patients were collected retrospectively from pediatric hematology and oncology centers in Turkey. A total of 65 patients (median age at diagnosis 17 months, range 2-117 months) were enrolled into the study. The median time from diagnosis to HSCT was 9 months (range, 2-63 months). The frequent reason for not performing HSCT was the inability to find a suitable donor. The median time from diagnosis to HSCT was 9 months (range 2-63 months). 32 out of 65 patients (49%) had a median time from diagnosis to HSCT of 9 months (range 2-63 months). The median time from diagnosis to HSCT was 9 months (range 2-63 months).

Frequent reasons for not performing HSCT were the inability to find a suitable donor. The median time from diagnosis to HSCT was 9 months (range, 2-63 months). 32 out of 65 patients (49%) had a median time from diagnosis to HSCT of 9 months (range 2-63 months). The frequent reason for not performing HSCT was the inability to find a suitable donor. The median time from diagnosis to HSCT was 9 months (range 2-63 months).

Background: JMML patients in Turkey, in terms of time of diagnosis, clinical characteristics, mutational studies, clinical course and treatment strategies.

Aims: To define the status of juvenile myelomonocytic leukemia (JMML) patients in Turkey, in terms of time 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: Seventy-five children with JMML diagnosed between 2002 and 2016 in 18 Turkish hospitals were enrolled into this 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 32.9 x 10^9/L, 5.4 x 10^9/L and 58.3 x 10^9/L, respectively. Monosomy 7 was present in 18% of patients. JMML mutational analysis was performed in 32 patients (29%) and 8% (54/66) of patients had the p.T501I mutation. Hematopoietic stem cell transplantation (HSCT) could only be performed in 28 (44%) patients, majority of being after the year 2012. The most frequent reason for not performing HSCT was the inability to find a suitable donor. The median time from diagnosis to HSCT was 9 months (range, 2-83 months). The 5-year cumulative survival rate was 33% and median estimated survival time was 30±17.4 months (95% CI 0-64.1) for all patients. Survival time was significantly better in the HSCT group (log-rank p=0.019). Older age at diagnosis (>2 years), platelets less than 40×10^9/L and age at diagnosis >12 months were independent risk factors for survival. In summary, our cohort of patients with MDS, MDS/MPN and sAML show clearly decreased survival levels. The preliminary results suggest a tendency of serum levels to increase with higher risk MDS/sAML which is supported by positive Kendall's tau (p=0.210). Serum OCN levels lie below normal limits, but seem not to be affected by disease risk. 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. In summary, our cohort of patients with MDS, MDS/MPN and sAML show clearly decreased survival levels. The preliminary results suggest a tendency of serum levels to increase with higher risk MDS/sAML which is supported by positive Kendall's tau (p=0.210). Serum OCN levels lie below normal limits, but seem not to be affected by disease risk.

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 (VL/L) and 34 patients with intermediate risk (INT) in the past 10 years in a single institution in Spain. In all 48 patients with IPSS-R very low/low risk (VL/L) and 34 patients with intermediate risk (INT) in the past 10 years in a single institution in Spain. We reviewed the percentage of PBC CD34+ (%PBC CD34+) in all 48 patients with IPSS-R very low/low risk (VL/L) and 34 patients with intermediate risk (INT) in the past 10 years in a single institution in Spain. We reviewed the%PBC CD34+ (CD34+CD10+ or CD34+CD117-) of these patients with a%PBC above the median had a median PFS of 2.4 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).
patients. All patients required erythropoiesis stimulating agents and 9 patients received treatment with azacytidine (AZA). In 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. In the AZA group, overall survival (OS) in patients with high risk disease was 67% (95% CI 55-77%), compared to 42% (95% CI 25-60%) in the non-AZA group. The most frequently mutated genes were related to signal transduction pathways (n=11; JAK1, JAK2, NRAS, CBL, GATA2, SH2B3, CSFR). Patients with mutations had significantly lower serum EPO levels (p < 0.001; median 32.3 vs 42.70 U/L). Furthermore, patients with such mutations demonstrated a clear discrepancy in transplant analysis, with a median OS of 19 months for patients who reached in patients without mutations (p<0.01), 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: Our data confirms 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 clear survival disadvantage across all risk groups of the IPSS and R-IPSS. This unveils the question whether we may be facing a shift towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.

PB1930

CLINICAL FEATURES, CYTOTOGENIC STUDY AND OUTCOME OF ADULT MELODYSLASIS 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 scored, including karyotypic, metaphase, and interphase FISH using a panel including six probes (5q-,7q-,20q-, del(17p13), MLL, inv(3) t(3;3). Patients were stratified into risk groups according to IPSS and IPSS-R scores; survival probabilities were estimated using the Kaplan-Meier method.

Results: Among these 101 pts, 58 were male with a sex ratio=1.35; range in age is from 18 years to 94 years with a median of 61, 6 years. Median hemoglobin value was 80 g/L (29-150), more than 60% of patients had severe anemia mainly in the low risk group; the median absolute neutrophil count was 3 G/L (0.06-13.5), and the median platelet count was 144 G/L (5-659). Median bone marrow blast count was 4% (0-18). Classes were classified by cytology morphology FAB classification (n=34), RARS (n=18), CMML (n=8), PASH (n=1), RAEB (n=1), RAEB-1 did not receive any therapy pre-transplant. However, both of them are AML-like, cytarabine and anthracycline based, intensive chemotherapy (I.C.). In selected cases fludarabine and cytarabine containing regimens are also used. In the last ten years, in the context of a clinical trial, a series of patients have received a less intensive, hypomethylating therapy (repeated courses of 5-azacytidine 75 mg/m2 subcutaneously for 7 days), as bridge to transplant. Conditioning regimens used in MDS patients is busulfan based in younger patients (Bu-Flu, BU-Cy); in the elderly or less fit patients a RIC regimen (thiotepa 5 mg/kg e.v., fludarabine mg/m2 x 3 and L-PAM 100 mg/m2) is administered.

Results: In the last ten years we performed 14 HCT (between June 2008 and September 2012) in patients with MDS in our institution. Median patient age was 63.5 years (range: 49-69), male/female ratio was 9/5. According to IPSS, 12 out of 14 patients were high-int2 (2 int-1), 11/14 had >10% blasts cell (BE-2). According to our centre protocol, we treated 11 patients with EB-2 and 1 patient with EB-1 (with hypercellular bone marrow) with a debulking therapy. This was I.C. in 6 patients and 5-AZA in 6 patients. Two patients with EB-1 did not receive any therapy pre-transplant. However, both of them are not evaluable, due to early mortality. Transplant conditioning was RIC in 11/14 patients, myeloablative in 3 cases. The donor was a sibling in 9/14, MUD in 5/14. Four out of six patients treated with I.C. achieved a pre-transplant CR (83.3%), comparable to one out of six in the 5-Aza cohort (17%). Four patients who reached 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 (CR vs 5-Aza, p=0.54), nor by pre-transplant disease state (CR vs nCR, p=0.22). In fact, 3 out of 6 patients treated with I.C. relapsed, but only 1 out of 6 treated with 5-Aza relapsed after transplant. Three out of four patients who subsequently relapsed had received RIC transplant; type of transplant was not associated with relapse (P=1.0). The only variable that showed a trend for reduced RR was MUD transplant (p=0.08).

Summary/Conclusions: Extreme caution must be used in considering our data, given the very small patients number. In our cohort, pre-transplant intensive debulking chemotherapy, although obtained an high rate of CR, showed no effect in preventing relapse. Larger studies are necessary to assess the real utility of I.C. in this subset of frail patients.

PB1932

IRON CHELATION THERAPY IN MYELODYSPLASTIC SYNDROMES AND IN OTHER TRANSPLANT-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: Our results are in agreement with those previously published regarding demographic features, histological analysis of peripheral blood (PB) and marrow aspirates or bone marrow biopsies.

Summary/Conclusions: This unveils the question whether we may be facing a shift towards the molecular level in MDS risk stratification and if therapies targeted to such molecules may improve the outcome of these patients.
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 <5000, 13 pts (18.8%) showed an increase of SF <500, in spite of ICT, and 18 pts (26.1%) showed an increase of SF ≥5000. 12 pts (17.4%) achieved a SF value <1000, and 48 pts (69.6%) a SF value <2500. Adverse events possibly related to DFX were observed in 30 pts (43.5%): renal (increase of serum creatinine): 14 pts (20.3%) (grade >2: 1 pt; 1.4%); gastrointestinal : 14 pts (20.3%) (grade >2: 1 pt; 1.4%); cutaneous: 2 pts (2.9%) (grade >2: no pts). Permanent discontinuation of ICT: 40 pts (58%), because of toxicity (16 pts: 23.2%, worsening of clinical condition (6 pts: 8.7%), discontinuation of transfusions (9 pts: 13%), allogeneic transplantation (9 pts: 13%). 5 pts (7.2%) (4 MDS and 1 PRCA) (with DFX: 4 pts; with DFO: 1 pt) showed an erythroid response following ICT, after 2, 4, 7, 32 and 112 months, respectively, and one of them (with PRCA) achieved complete remission. 30 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.

Summary/Conclusions: In conclusion, in our experience ICT appears feasible both in terms of reduction of SF and OS, even in a population of elderly patients over malignant plasma cells and the molecular changes produced for the different reasons each other.

Methods: Interactions between both cell types were studied through different co-cultures studies. We evaluate differences between culturing primary MSC and MM cell line RPMI 8226. Pathological MSCs were extracted from the bone marrow of newly 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 proteins (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-apoptotic 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.

PB1934

RALA AND RALB MEDIATE CELL SURVIVAL INDEPENDENTLY OF ONCOSCENIC RAS AND PROVIDE POTENTIAL THERAPEUTIC TARGETS IN MULTIPLE MYELOMA

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Background: Genetic mutations and the bone marrow microenvironment contribute to disease progression, aggressive phenotype, and shorter survival in multiple myeloma (MM). Oncogenic RAS is one of the most common mutations in MM. Pathway activation through oncogenic RAS is associated with promotion of disease progression and shorter survival. Cell survival and proliferation in MM are mainly mediated via classical signaling pathways such as MEK/ERK and PI3K/Akt. Since there is a lack of specific RAS-inhibitors for clinical use, it is important to identify and analyze associated pathways, which may provide useful alternative targets for MM therapy. The small GTPase Ras has previously...
been implicated in putative downstream signaling of RAS, and may therefore promote tumor proliferation and survival of MM cells.

**Aims:** We used shRNA-mediated knockdown of RalA and RalB isoforms to appraise their role as potential therapeutic targets and to analyze their contribution to important signaling pathways, which regulate MM cell survival and proliferation. Because oncogenic RAS is a potential activator of the Ral pathway, we investigated the underlying role of Ral in oncogenic RAS-dependent pathways.

**Methods:** Immunohistochemical stainings of bone marrow trephines of MM patients and Western analysis of primary MM cells and MM cell lines were performed to evaluate Ral protein expression. Transient or stable knockdown of RalA or RalB was achieved by electroporation of MM cell lines and the effect on proliferation was measured with flow cytometry using annexin V/propidium iodide staining. Ral pulldown assays were applied to test functional dependence of Ral activation on oncogenic RAS. Furthermore, RNA sequencing was performed to compare RAS and Ral gene expression signatures after respective knockdowns.

**Results:** Both Ral variants were expressed in primary MM cells and MM cell lines, with RalA showing the most prominent and consistent protein expression levels. shRNA-mediated knockdown of RalA strongly induced apoptosis in two thirds of the tested cell lines, whereas RalB depletion did not impair MM cell survival in less than half of the cell lines. Western analysis revealed no alteration of classical Ral effectors and downstream pathway activation after Ral knockdown. Ral activity appears to be independent of oncogenic KRAS or NRAS. In addition, RNA sequencing revealed differing gene expression signatures for RAS and Ral.

**Summary/Conclusions:** Ral and its effector network constitute potential therapeutic targets in MM, which are activated independently of oncogenic K- or NRAS. Therefore, investigation of the functional network of Ral may be important to identify useful clinical targets. 

PB1935

**CXCR4 MUTATIONS FOUND BY USING DEEP SEQUENCING WITHOUT SORTING B CELLS, AND PROGNOSTIC IMPLICATION IN WALDENSTROM MACrogLOBULINEMIA**


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**Background:** Waldenstrom macroglobulinemia (WM) is a lymphoplasmacytoid lymphoma with IgM monoclonal 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 mutations, and its clinical implication is not reported in Asian patients with WM.

**Results:** 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.

**CXCR4**

- **Mutation:** Two variants, K327X and K331X, are novel and the rest were known reported mutations. All of them had significant (p=0.024). Mean IgG level was significantly lower in group 1, compared to group 2 (94.7%), though it was not statistically significant (p=0.155). There was no correlation among cytogenetic aberrations and genetic mutation.

**Summary/Conclusions:** The frequency of CXCR4 mutation in Korean WM was similar to those of Caucasian. We suggest that ultra-deep sequencing using whole-genome sequencing can detect and evaluate the prognostic implication of CXCR4 mutation. Patients with MYD88 WT and CXCR4 WT showed higher IgM level and lower survival, suggesting an adverse prognostic implication. This is the first report on CXCR4 mutation in Korean WM patients.

PB1936

**THE CLINICAL IMPACT OF CHROMOSOMAL TRANSLOCATION t(14;16)(q32;q23) IN PATIENTS WITH MULTIPLE MYELOMA**

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**Background:** Translocation t(14;16)(q32;q23) in plasma cells is considered as a strong negative prognostic factor in patients with multiple myeloma (MM). The oncogenic potential of this chromosomal aberration is based on the overexpression of the c-MAF protooncogene (located at 16q23) under strong enhancer of IgH gene (14q32). Although the IgH/MAF positive cases comprise just 2-4% of MM patients, the evaluation of this aberration is an integral part of the cytogenetic risk stratification according the RISS. The translocation (t(14;16)) is a hallmark of Myeloma Workshop (MYW) MM series. Its role as a strong negative prognostic factor in MM as having at least one of the following aberrations: deletion of 17p13 (TP53 gene), translocation t(14;16)(q32;q23) and translocation t(14;16)(q32;q23) determined by FISH. However, the unequivocal poor prognostic value of t(14;16)(q32;q23) was not confirmed in several MM series thus further studies are needed.

**Aims:** The aim of our study was to assess the impact of t(14;16)(q32;q23) on event free (EFS) and overall survival (OS) in cohort of IgH/MAF positive MM patients in comparison with control group of 30 MM IgH/MAF negative cases.

**Methods:** During the years 2004 to 2016, we examined 870 bone marrow samples of MM patients on immunofluorescently labeled plasma cells (clg FISH). The basic FISH panel included 4 specific DNA probes (Abbott-Vysis, Kreatech and MetaSystems) detecting: the IgH gene rearrangement (1), deletion 13q14 (RBB1 gene/monosomy 13 (2), gain of 1q21/deletion of 1p32 (3) and deletion of TP53 gene (4). Cases with rearranged IgH gene were gradually examined for 3 specific translocations: t(11;14)(q13;q32), t(4;14)(p13;q32) and t(3;14)(q14;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 (t14;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.

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Figure 1.
PB1937
THE ROLE OF NEUROTROPHINS AND ANGIOGENIC CYTOKINES IN THE PATHOPHYSIOLOGY OF PERIPHERAL NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA
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Background: The introduction of new treatment modalities have changed significantly the prognosis of multiple myeloma (MM) patients. The novel drugs and schemes of treatment of MM have contributed to substantial extend of the overall survival time of patients. However, the administration of some of the treatments, e.g. thalidomide or bortezomib 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/or 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 VMP or VTD. The control group consisted healthy age-matched subjects. Assessment of concentrations of neurotrophins (BDNF, NSE) and angiogenic factor (PDGF) were performed using Luminex technology, which utilize microbeads coated with fluorescently labeled antibodies.

Results: Concentration of BDNF, PDGF and NSE were 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-kB during the treatment of MM, since reduction of NF-kB concentration is associated with decrease in the transcription of genes encoding BDNF, NSE and PDGF.

Summary/Conclusions: Alterations in the concentration of BDNF, PDGF and NSE suggest the cause and effect relationship between these factors and the development of neuropathy in patients with MM. Comprehensive elucidation of this phenomenon may contribute to the extension of the knowledge concerning the pathogenesis of neuropathy, and might well lead to reduction of the incidence of polyneuropathy in MM patients in the future.

PB1938
INFLUENCE OF XRCC5, XRCC4, NFKB2, AND BIRC5 GENES PolyMorphISmS IN THE RISK AND PROGNOSIS OF MONOCLONAL GamMOpathIes L. Balanço1, M.S. Melo1, A.C. Gonçalves1,2, R. Alves1,2, C. Geraldes1,2,3, R. Alves1,2, C. Geraldes1,2,3, R. Alves1,2, C. Geraldes1,2,3, R. Alves1,2, C. Geraldes1,2,3
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Background: Monoclonal gammopathies (MG) are a group of disorders characterized by the proliferation of monoclonal plasma cells, which produce and secrete monoclonal immunoglobulin (M protein). Symptomatic multiple myeloma (MM) are characterized by the clonal proliferation of plasma cells. MM is consistent preceded by a pre-neoplastic entity, called monoclonal gammopathy of undetermined significance (MGUS), with an intermediate phase of indolent multiple myeloma (MMi). This disease is a heterogeneous hematological neoplasm characterized by the proliferation of clonal, long-lived plasma cells within the bone marrow (BM) secreting monoclonal proteins and by the presence of so-called CRAB criteria and/or biomarkers of malignancy (as clonal BM plasma cells > 10%, involved/uninvolved serum free light chain ratio >100, >1 focal lesion in MRI studies). Genetic instability and several molecular abnormalities are hallmarks of MM cell lines. Alterations in DNA repair pathways, namely abnormal activity of non homologous end-joining (NHEJ) repair pathway, are involved in the disease onset and progression. Moreover, it has been observed that virtually all primary MM samples have constitutive nuclear factor-kB (NF-kB) pathway activity, having this pathway a well-established role in MM pathogenesis. Therefore, this study aimed to investigate the association of SNPs in genes involved in NHEJ repair pathway (XRCC5, XRCC4) and in NF-kB pathway (NFKB2, and BIRC5) may impact in MG susceptibility and prognosis.

Methods: In the present, a hospital-based case-control study, we analyzed eight polymorphism in four genes (XRCC5, XRCC4, NFKB2, and BIRC5), by genotyping 189 individuals (63 MG patients and 126 controls) using TaqMan qPCR. Results are expressed in terms of frequencies of allele, genotype, haplotype, and genotypic profiles, and their correlation with MG susceptibility. The strength of association between polymorphisms and disease risk was assessed by odds ratio (OR) with 95% confidence interval (CI95%) calculated by logistic regression. We also investigated the association of these SNPs with overall survival through Kaplan Meier curves. All statistical analyses had a significance levels of 95%.

Results: In the patient group, 51% (32/63) of the individuals were females and 49% (31/63) were males; the mean age was 70.1±10.25 years old. Among the 63 patients, 52% (65/126) of the individuals were females and 48% (61/126) were males, and the mean age was 69.9±10.06 years old. Most of patients were diagnosed with multiple myeloma (84%), 53/63 and the remaining ones (16%, 10/63) were diagnosed with smoldering multiple myeloma. According to the ISS classification, 43% (27/63) of patients are in stage III. The data analysis revealed two associations of the studied gene polymorphisms with MG. First, the analysis by gender stratification suggested a decreased predisposition to MG in male carriers of NFKB2 rs12769316 GA and AA genotypes (OR 0.346, 95%CI 0.124–0.965, p=0.043). Second, we observed that patients with BIRC5 rs9904341 CC genotype had a highly significant lower overall survival (recessive model: HR 4.89, 95%CI 5.06 199.70, p<0.01). BIRC5 GGC haplotype (rs4789551, rs9904341, and rs8073069) was found in one patient and absent in controls.

Summary/Conclusions: The present study suggests that NFKB2 gene variant (rs12769316, allele A) may be associated with MG susceptibility in males, and BIRC5 (rs9904341 CC genotype may negatively influence MG prognosis. Nonetheless, further studies are needed to validate these findings, enlighten the role of genetic polymorphisms in MG susceptibility and prognosis.

PB1939
SILENCE OF LONG NONCODING RNA MALAT1 BY RNA INTERFERENCE INHIBITS PROLIFERATION AND INDUCES APOPTOSIS IN MULTIPLE MYELOMA H. Liu1,2, Z. Gong1,2, W. Yang1
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Background: Multiple myeloma (MM) is a neoplastic plasma-cell disorder characterized by abnormal proliferation of monoclonal plasma cells in bone marrow leading to various end-organ damages. Altered long non-coding RNAs (lncRNAs) levels can result in aberrant expression of gene products that may contribute to cancer biology. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), an evolutionarily highly conserved mRNA-like IncRNA was originally identified with high expression in metastatic non-small-cell lung cancer and reported to be up-regulated in many other cancers. However, the function of MALAT1 in MM remains unknown.

Aims: Our study aimed to evaluate the role of MALAT1 on proliferation as well as apoptosis in MM cells in vitro and tumorigenic ability in vivo, following transcription with MALAT1-specific short hairpin RNA (shRNA) expression plasmids.

Methods: Levels of MALAT1 in MM were investigated by real-time polymerase chain reaction (RT-PCR) analysis. The effects of MALAT1 shRNA in MM were investigated in vitro and in vivo.

Results: We found that MALAT1 was high expressing in RPMI8226 and U266 cell lines. Inhibition of MALAT1 by shRNA significantly inhibited the proliferation through cell cycle arrest at G1 phase and induced apoptosis, which was closely associated with activation of caspase-3/9, downregulation of Bcl-2 and upregulation of Bax. Study in vivo revealed that silencing of MALAT1 delayed the tumor growth and led to apoptosis in mice bearing xenograft.

Summary/Conclusions: MALAT1 may serve as a promising novel therapeutic target in human MM. Notably, the inhibition of MALAT1 by shRNA may prove to be an effective genetic therapeutic strategy for MM treatment.

PB1940
LONG NON-CODING RNA MEG3 FUNCTIONS AS A COMPETING ENRyTE (ceRNA) To REGuLATE PTEn EXPRESSION BY SPONGING MIR-181A IN MULTIPLE MYELOMA L. Chen1, H. Bai1, H. Zhu1, X. Shen1, J. Li1, W. Yu1
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Background: Long non-coding RNA maternally expressed gene 3 (MEG3) plays a critical role in cancer progression and metastasis. However, the overall biological role and regulatory mechanism of MEG3 in multiple myeloma (MM) development and progression remains largely unknown.

Aims: To explore the tumor suppression role of IncRNA MEG3 in MM and further reveal the mechanism of MEG3 functions as ceRNA to contribute to MM pathogenesis.

Methods: MEG3 expression was measured in MM patients by real-time PCR.
PB1941

IMPRESSIVE 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-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 detection rate 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 of IgH rearrangement, del(13q14), del(17p) and 1q21 gains rose to 56.3% (P<0.001), 37.5% (P<0.001), 22.9% (P<0.001) and 41.7% (P<0.001), respectively, all significant increases compared to untreated samples.

Summary/Conclusions: We have developed a rapid, simple assay for improved diagnostics and risk-stratification for MM. With more precise diagnoses, the clinical outcomes of MM will be significantly improved.

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SERUM FREE LIGHT CHAIN RATIO IS AN INDEPENDENT RISK FACTOR FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

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Background: Monoclonal gammapathy of undetermined significance (MGUS) is a premalignant plasma cell proliferative disorder found in approximately 3% of the general population 50 years of age and older. MGUS is associated with progression to multiple myeloma or related malignancy at a rate of 1% per year. Thus the risk of malignancy for a 50-year-old patient with a 25-year life span is 25%.

Aims: We hypothesized that the presence of monoclonal free kappa or lambda immunoglobulin light chain in monoclonal gammapathy of undetermined significance (MGUS), as detected by the serum free light chain (FLC) assay increases the risk of progression to malignancy.

Methods: 90 Patients seen at the Hematology consultation from 2010 to 2015 with MGUS have a serum Mprotein less than 30 g/L, bone marrow plasma cells less than 10%, and no anemia, hypercalcemia, lytic bone lesions, or renal failure that would be indicative of a malignant plasma cell disorder.

The prognostic effect of abnormal kappa-to-lambda FLC ratio on progression of MGUS was studied. We also examined whether the risk of progression varied depending on the extent to which the FLC ratio was abnormal (the normal distribution range of k/λ ratio 0.25 to 1.65).

Results: The median age at diagnosis of MGUS was 59 years (35-92years). 62 Womans and 28 Mans Sex ratio=2.2. Serum electrophoresis and immunoelectrophoresis or immunofixation was done in 85 patients. Of these, The median serum M protein size at diagnosis was 12 g/L (1.7-28.5 g/L). IgG monoclonal - 68% (56 patients), and non IgG monoclonal - 22 patients (25%). A monoclonal light chain was detected in 62 patients, as detected by the serum free light chain (FLC) assay increases the risk of progression to malignancy. An abnormal FLC ratio (kappa-lambda ratio <0.26 or >1.65) was detected in 27 (30%) patients. At a median follow-up of 5 years, malignant progression had occurred in 6 patients (6.6%) with an abnormal serum FLC ratio.

Summary/Conclusions: A novel, highly sensitive serum free light chain (FLC) assay is now available for clinical practice. The risk of progression in patients with an abnormal FLC ratio was significantly higher compared with patients with a normal ratio, and was independent of the size and type of the serum monoclonal protein. In conclusion, the serum free light chain (FLC) promoted cell apoptosis and inhibited cell proliferation, migration and angiogenesis in MM ARP1 and LP-1 cell lines. Furthermore, MGUS increase the expression of phosphatase and tensin homolog (PTEN) and consequently inhibit MM cell proliferation and angiogenesis through sponging miR-181a in a manner not limited by PTEN by transcription.

PB1943

INTENSITY OF EXPRESSION OF MULTIDRUG RESISTANCE GENES AFFECT ON THE OVERALL SURVIVAL OF PATIENTS WITH MULTIPLE MYELOMA WHO WERE TREATED WITH BORTezOBIb AND ASSOCIATED WITH THE INITIAL MULTIDRUG RESISTANCE

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Background: Bortezomib is an important drug in multiple myeloma (MM) treatment, but the resistance to this treatment exist. Many conflicting data suggests that cellular overexpression of multidrug resistance (MDR) genes may reduce the effectiveness of bortezomib - containing treatment. The main indicator of the effectiveness of the treatment of MM is the overall survival of patients.

Aims: We evaluated the changes of intensity of expression of MDR genes in patients with newly diagnosed and refractory/relapsed multiple myeloma and the effect of expression of MDR genes such as MDR 1, MRP 1, BCRP, LRP on the overall survival of patients after treatment with bortezomib.

Methods: We studied 3 groups of patients. The group of 30 men (12 men and 18 women) aged 48 to 77 years (median 60 years) with stage III MM by classification Durie-Salmon were studied. 15 patients were included in a group of newly diagnosed (ND) MM. 15 patients were in group of a clinically refractory/ relapsed (RR) MM. The bone marrow in this group of patients were studied after treatment with alkylating agents at the time of registration of resistance to the given therapy. In the future, all patients were treated by bortezomib - containing chemotherapy regimen. mRNA expression studied genes were determined by semi-quantitative polymerase chain reaction reverse transcription. The degree of expression was assessed by semi-quantitative visual assessment from 0 (no photorethoric dots) to 4 points (bright photorethoric dots). The overall survival (OS) was analyzed by the Kaplan-Meier method, with the use of Cox-Mantel test. Differences were considered statistically significant at p<0.05.

Results: In both groups of patients had comparable expression of all studied MDR`s genes. The development of clinical resistance to treatment with alkylating agents were accompanied by an increase in mRNA expression of all studied genes. However, the statistically significant increase the expression of the intensity obtained for LRP gene only (the average intensity of the expression of mRNA LRP gene in ND MM 0.9±0.24, with RR MM 1.93±0.34, p<0.05). The MDR 1 mRNA expression was 1.50±0.34 in the group of ND MM and 1.67±0.31 in the group of RR MM, p<0.05. The expression of mRNA of MRP 1 and BCRP were 1.07±0.21 and 1.63±0.15 respectively before treatment and increased to 1.73±0.31 and 2.13±0.35 respectively in the group of RR MM, p=0.06. OS was negatively associated with high LRP gene expression only in group of ND MM (median of OS in patients with high LRP gene expression was 5 months and in patients with low LRP gene expression was 15 months).

Summary/Conclusions: High expression of LRP gene is associated with worse overall survival in patients with newly diagnosed MM treated with bortezomib containing chemotherapy programs. "Genetic resource MDR" in MM is due mainly to the initial multidrug resistance. The treatment of MM by alkylating drugs increase the existing at the time of diagnosis of MDR activity of genes.

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ASSOCIATIONS OF IL-1, IL-4 AND TGF-B1 POLYMORPHISMS WITH CYTOTGENIC PROFILES IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is a plasma cell malignancy characterized by complex cytogenetic and molecular genetic aberrations. These cytogenetic abnormalities occur at different stages of the disease. The chromosome ploidy status and Ig rearrangements are two genetic criteria that are used to help stratify patients into prognostic groups.

Aims: The aim of the study was to analyze correlation between cytogenetic profiles and some cytokine genotypes in 24 patients with MM (Caucasoid inhabitants of the North-West region of Russia).

Methods: Genomic DNA was extracted from the peripheral blood; gene geno-
typing (IL-4, TGF-β1, IL-1α, IL-18) was performed by PCR-SSP; study of cytoketic abnormalities was performed by standard GTG-method and interphase FISH analyses with DNA probes: LSI 13(RB1)13q14, IGH/CCND1, IGH/FGFR3, LSI TP53 (17q13.1); p-values less than 0.05 were considered statistically significant. Results: Previous results allow us to describe some cytoketype marker combinations associated with the development of MM (IL-1α -889 TT, IL-1β -3962 TT, IL-6 -174 GG and IL-6 n565 G) 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 cytoketric profiles in MM patients with different prognostic markers in their genotypes (Table 1).

The frequency of abnormal cytogenetic transformations in the 2nd gr. was noticeably lower compared to patients from the 1st and 3rd gr. (0.11 vs 0.87 vs 0.67 respectively; p<0.05). Similarly, significant differences in the frequency between patients with positive prognostic markers and normal cytogenetic profile (0.89) compared to MM patients negative (0.22) or mixed (0.33) genotypes but normal cytogenetic profiles were also observed (p<0.05). In the 1st gr. frequency of cytogenetic abnormalities was noticeably higher compare to patients with normal profile (0.78 vs 0.22; p<0.05). Vice versa, in patients with positive prognostic markers the frequency of normal cytogenetic profiles was remarkably higher (0.89) compared to patients with aberrations (0.11; p<0.05).

Summary/Conclusions: Thus, our results allow to describe IL-1α -889 TT, IL-1β -3962 TT, IL-6 -174 GG and IL-6 n565 G as markers associated with the presence of cytoketic abnormalities in MM patient cells. However, IL-4 -33 CC and TGF-β1 codon 25 GG genotype and normal cytogenetic 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 cytoketic abnormalities is much higher compare 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. Study of 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 the development of chronic lymphoproliferative disorders (CLD) and multiple myeloma (MM). It is noted that in the pathogenesis of hematological diseases contribute significantly to certain combinations of immune response genes.

Aims: Analysis of interactions between genes based on the distribution of immune response gene combinations in chronic lymphoproliferative disorders and multiple myeloma. Methods: The study included 176 patients aged 22-86 years (median - 61 year), identifying themselves as Caucasians residing in one region in the north-east of the Russian Federation. This group consisted of 80 patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (45%), 72 with multiple myeloma (41%), 10 with diffuse large B-cell lymphoma (6%) with marginal zone lymphoma (3%) four with mantle cell lymphoma (2%), three with lymphoplasmacytic lymphoma (2%) and one patient with follicular lymphoma (1%). Genotyping of polymorphism of the innate immune response genes TRLR2 (rs5743708), TLR3 (rs3775291), TLR6 (rs5743810), TLR9 (rs5743836), IL1β (rs2856841), IL2 (rs2069762), IL4 (rs2243250), IL6 (rs1800795), IL10 (rs3935827), TNFAIp6 (rs34424920), TNFα (rs3003588), FCGR2A (rs1801274) were performed by polymerase chain reaction with allele-specific primers (Litech, Russia). Analysis of interactions between genes was performed using nonparametric GMDR program (Generalized Multifactor-

Dimensionality Reduction) [Luo X.Y. et al., 2007, http://www.healthsystem.vir

ginia.edu/internet/addiction-genomics/Software/].

Results: In the analyzed group of patients with CLD and MM identified almost 78 753 combinations of multi-locus genotypes of the 13 immune response genes is 1 594 323 theoretically possible, indicating the non-random nature of the combination of allele variants of analyzed genes. A statistically significant two-, three-, four-, five-, six-, seven- and eight-loci model of inter-gene interactions at the investigated hematological malignancies: - IL4 (C-589T) and CD14 (C-159T) (χ2=8.39, p=0.0038); - IL4 (C-589T) and CD14 (C-159T) and IL6 (C-174G) (χ2=12.14, p=0.0005); - IL4 (C-589T) and IL17A (G-197A) and CD14 (C-159T) and IL6 (C-174G); - IL4 (C-589T) and IL17A (G-197A) and L10 (C-819T) and CD14 (C-159T) and IL6 (C-174G) (χ2=16.98, p<0.001); - IL4 (C-589T) and IL17A (G-197A) and IL10 (C-819T) and TNFα (C-308A) and CD14 (C-159T) and IL2 (T-330G) (χ2=16.98, p<0.001); - IL4 (C-589T) and IL17A (G-197A) and IL10 (C-819T) and TNFα (C-308A) and CD14 (C-159T) and IL2 (T-330G) and IL1b (T-31C) (χ2=16.98, p<0.001); - IL4 (C-589T) and IL17A (G-197A) and IL10 (C-819T) and TNFα (C-308A) and CD14 (C-159T) and IL2 (T-330G) and IL1b (T-31C) and TLR9 (T-2373C) and CD14 (C-159T) and IL2 (T-330G) and IL1b (T-31C) and TLR2 (Arg753Gln) (χ2=16.98, p=0.001).

Summary/Conclusions: The findings suggest an important role of immune response genes in the development of a number of chronic lymphoproliferative disorders and multiple myeloma, and can later be used as diagnostic and prognostic markers of different types of hematological malignancies. In addition, the cytoketic polymorphisms remarkable not only for the high and low risk of hematological malignancies studied, but also to determine their prognostic significance in the clinical course of these diseases.

PB1946

FEATURES OF STROMAL ELEMENTS IN HEMATOPOETIC BONE MARROW NICHE IN MULTIPLE MYELOMA

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Background: Structure of bone marrow stroma — mesenchymal stromal cells (MSC), endostal stromal cells, and microvessels forming the hematopoietic niche and regulate the development of hematopoietic stem cells (HSC). Analysis of morphological changes of these elements of the hematopoietic niche is important to clarify the pathogenesis of multiple myeloma (MM). Aims: To investigate the morphological and functional characteristics of stromal elements of the hematopoietic niche in bone marrow of patients with MM, as well as the characteristics of culture of mesenchymal stromal cells (MSC) and hematopoietic stem cells (HSC).

Methods: 42 trepanobiopsy of bone marrow from patients diagnosed with MM were used for the study. The age of the patients ranged from 53 to 72. The study applied histological, histochemical, immunohistochemical (IHC) and morphometric methods (VideoTest®). Also 20 patients from this group conducted cultural studies for the determination of colony-forming ability of HSC and morphokinetic status of MMC.

Results: Myeloma cellular composition of infiltrates were polymorphic. The surveyed patients were allocated to 3 types of infiltration is nodular, interstitial, diffuse. The histogenesis of infiltration was confirmed by IHC research with antibodies 7B9, CD 138, CD 38.Regardless of the type of infiltration in all patients were recorded focal destructive changes of bone tissue. The density of microvessels N.Y. Semenova1, S. Bessmeltsev1, V. Rugal1

et al. Madrid, Spain, June 22 – 25, 2017

haematologica | 2017; 102(s2) | 775

summary/Conclusions: Analysis of parenchymal-stromal relationships in trepanobiopsy bone marrow of patients with MM evidence of their violation in the context of malignancy of lymphopoesis, while cultural studies have shown a decrease of colony-forming ability of HSC and proliferative capacity of MSC. Features of the prevalence of neoplastic lesions, and myeloma infiltration noted the response of the stromal microenvironment, forming the hematopoietic niche.

PB1947

Abstract withdrawn.
Myeloma and other monoclonal gammopathies - Clinical

PB1948
Abstract withdrawn.

PB1949
IMPACT OF RENAL IMPAIRMENT IN NEWLY DIAGNOSED MULTIPLE MYELOMA IN A REAL WORLD SETTING

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Background: Renal impairment (RI) is a frequent complication of patients with newly diagnosed multiple myeloma (NDMM), reported in 15-40% with 10% requiring hemodialysis (HD). It is associated with higher early mortality (EM) and lower overall survival (OS). Early diagnosis and treatment with new agents improve these results.

Aims: Analyze renal response, OS and EM in NDMM with RI and compare them to patients with MM without RI.

Methods: All consecutive and unselected NDMM patients treated at Hospital de Clínicas, Montevideo, Uruguay, from January 2011 to June 2015 were included. Our database was completed prospectively and included clinical and laboratory characteristics of the disease, treatment, treatment-related adverse events, response, HD requirement, renal response and mortality.

Diagnosis of MM, response to treatment and degree of renal function recovery were based on the International Myeloma Working Group criteria. RI was defined as an estimated glomerular filtration rate (eGFR) <40 ml/min/1.73m², calculated by MDRD (Modification of Diet in Renal Disease) equation. Patients whose RI was explained by other causes were excluded. Early treatment was defined by initiation within 7 days after diagnosis. EM was defined as death within 3 months of diagnosis.

Results: MM was diagnosed in 52 patients, median age was 67 years (range 39-90), 61.5% were male, 38.5% had RI. The characteristics of the patients and front-line treatment are shown in Figure 1.

PB1950
THE EXPRESSION OF THE TRYPHTASE POSITIVE MAST CELLS AND THE LEVELS OF IL-17, CORRELATE WITH ANGIOGENIC FACTORS IN PATIENTS WITH MULTIPLE MYELOMA

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Background: Angiogenesis in the bone marrow plays a very important role in the progression of multiple myeloma (MM). The procedure of angiogenesis is stimulated by several factors such as VEGF, FGF-2 and metalloproteinases that are secreted straight from the tumor cells. The presence of IL-6 in the microenvironment, induces the production and the secretion of several angiogenic factors that activate inflammatory cells of the matrix, like macrophages and mast cells to secrete more angiogenic factors. IL-17 is among the most important cytokines that have an important role in the development of myeloma tumor. IL-17 is a proinflammatory cytokine that is secreted primarily by CD4 (activated memory cells) and stimulate macrophages, fibroblasts and other cells that release several cytokines. It has been reported that IL-17, induces angiogenesis in humans by stimulating the migration of vessel endothelial cells and adjusting the production of various proangiogenic factors. In a previous study, it was found that increased levels in stage II and stage III, resolved after therapy. Additionally, blocking the receptor of IL-17, with an antibody, cancels the effects of IL-17.

Aims: Aim of this study is to assess the relationship of the MCD and IL-17, in angiogenesis of MM, as well as their correlation with known angiogenic factors in disease progression.

Methods: We studied 52 newly diagnosed patients with MM, 32 women and 20 men, aged 67±9.6 years. According to the ISS stage, 19 were stage I, 17 stage II and 16 stage III. Regarding the type of paraprotein that had been found, 31 IgG, 17 IgA and 4 patients with light chains. 20 age and sex-matched healthy volunteers, were used as controls. Serum samples and bone marrow biopsy samples were obtained from all patients prior to the initiation of treatment. Patients with impaired hepatic and renal function, chronic or acute infections, chronic inflammatory or autoimmune disorders or other malignancies were excluded from the study. We also excluded patients who were taking anti-inflammatory drugs, corticosteroids or bisphosphonates. Serum samples were taken at MCD and 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 mast 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). 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 MCD cells increase in the bone marrow(BM) of patients with MM. They release several transmitters that promote direct and indirect the development of new angiogenesis. Progress of MM also accompanied by increased angiogenesis in BM. In conclusion, mast cells and angiogenic factors seem to be important elements in the development of MM and become potential targets for the treatment and prognosis of the disease.
PB1951
HEALTHCARE RESOURCE UTILIZATION ASSOCIATED WITH DIFFERENT TREATMENT MODALITIES OF RELAPSED/REFRACTORY MULTIPLE MYELOMA PATIENTS IN THE US: FINDINGS FROM PREAMBLE
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Background: Proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) and treatments involving both a PI and an IMID (PI+IMID) are the principal therapies for treating relapsed/refractory multiple myeloma (RRMM). The widespread adoption of these treatments may come with high healthcare resource utilization (HCRU), of which key drivers are reported in past research. It is important to further understand HCRU by different treatment modalities in real-world practice settings.

Aims: To evaluate HCRU in patients receiving different treatment modalities for RRMM.

Methods: US patients with RRMM, aged ≥18 y, with at least one prior therapy who initiated treatment with a PI, IMID or IMID+PI within 90 d before or 30 d after study enrollment (index therapy), were identified from PREAMBLE, an ongoing, prospective, multinational, non-interventional observational study. Patient data collected at each healthcare provider (HCP) visit, over a 3-y period or until the end of patient follow-up, included clinic/physician office visits; home healthcare, hospital outpatient and emergency room visits; and hospitalizations. Demographics and baseline characteristics were summarized using descriptive statistics. HCRU and its associated costs were analyzed using a standard per-1000 patients-per-month metric.

Results: 287 patients (median age 66 y; 56% male) were enrolled in the US. At the time of data cut-off (Sep 2016), 136 (47%) were still in the study and 151 (53%) had withdrawn; 92 (61%) of those withdrawn had died. Median (range) follow-up was 12.7 (0.5–41.0) mo. At study entry, patients were divided into three cohorts based on index therapy: PI (n=162, 56%); carfilzomib n=82/162; bortezomib n=80/162), IMID (n=74, 26%; pomalidomide n=32/74; lenalidomide/thalidomide n=42/74), and PI+IMID (n=51, 18%; carfilzomib and/or pomalidomide n=17/51; other n=34/51). The three groups were similar with regard to sex, race, disease status, ISS stage, comorbidities and number of prior therapies (Table 1).

Table 1. Patient characteristics and risk stratification.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PI (n=162)</th>
<th>IMID (n=74)</th>
<th>PI+IMID (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (y)</td>
<td>59 (42–78)</td>
<td>61 (45–75)</td>
<td>62 (47–73)</td>
</tr>
<tr>
<td>Male gender (%)</td>
<td>65%</td>
<td>71%</td>
<td>66%</td>
</tr>
<tr>
<td>ISS stage (%)</td>
<td>16 (4%)</td>
<td>26 (35%)</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Comorbidities (%)</td>
<td>78 (48%)</td>
<td>79 (53%)</td>
<td>80 (59%)</td>
</tr>
</tbody>
</table>

The median duration of treatment (mDoT) was longer for patients on IMID (6.4 mo), but shorter for those on PI (4.2 mo) or PI+IMID (4.4 mo). In the PI cohort, carfilzomib had a shorter mDoT than bortezomib (3.5 vs 4.7 mo). Of 3220 total HCP visits, the most common type was clinic/physician office (2732, 85%), followed by hospitalization (210, 7%) and hospital outpatient (54, 5%). Mean per-1000 patients-per-month total visits were higher for PI+IMID (876) than for PI (750) and IMID (494). This remained true for clinic/physician office, hospital outpatient and home healthcare/other. Patients on PI had more visits for management of MM treatment-related events (16%) than those on PI+IMID (10%) or IMID (7%) (Table 1). Notably, among patients on PI, those on carfilzomib had high mean per-1000 patients-per-month total visits (827), with per-1000 patients-per-month emergency room visits (18) and hospitalizations (78) higher than any other treatment; 19% (175) of visits were made for management of treatment-related events.

Summary/Conclusions: Routine management of MM and treatment-related events drive HCRU, which may differ by treatment. Hospitalizations and hospital outpatient visits remain key drivers of HCRU in MM, which highlights an unmet medical need for effective therapy with better safety profiles.

PB1952
ASSOCIATION OF SERUM HEAVY/LIGHT CHAIN PAIR SUPPRESSION WITH RISK FACTORS FOR PROGRESSION IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MYELOMA
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Background: Monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) are conditions that usually precede symptomatic multiple myeloma (MM). Risk stratification is crucial, considering the heterogeneous progression rate among these patients and the chemoprevention trials encouraged for high risk individuals. A number of prognostic factors for progression have been identified. In this sense, the novel Hevylite assay now enables us to accurately measure each isotype-specific heavy and light chain (HLC). Recently, isotype-specific uninvolved HLC pair suppression was described as an independent predictor of progression to MM in patients with MGUS. The role of Hevylite as a prognostic factor in SMM is less investigated.

Aims: The aim of the present study was to analyze the impact of HLC pairs in a series of patients with high risk MGUS and SMM and their relationship with other previously described risk factors.

Methods: Forty-four patients diagnosed with high risk MGUS or SMM at a single institution from March 2014 through April 2016 were prospectively included in the present study. Patients were stratified according to the Mayo Clinic and the Spanish PETHEMA group models. Samples at diagnosis were tested for HLC concentrations for the three pairs (IgG, IgM and IgA) by immunonephelometry.

Results: The clinical characteristics and risk stratification of patients are summarized in Table 1.

Table 1. Patient characteristics and risk stratification.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male (%)</th>
<th>Age (median, range)</th>
<th>Median MCV</th>
<th>IgG (g/L)</th>
<th>IgM (g/L)</th>
<th>IgA (g/L)</th>
<th>Hevylite</th>
<th>Mayo Clinic</th>
<th>PETHEMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (%)</td>
<td>24 (54%)</td>
<td>66 (46–87)</td>
<td>87 (78-104)</td>
<td>18 (10–35)</td>
<td>1 (0–4)</td>
<td>0.25 (0–1)</td>
<td>0.5 (0)</td>
<td>0.4 (0)</td>
<td>0.5 (0)</td>
</tr>
<tr>
<td>ISS stage (%)</td>
<td>23 (52%)</td>
<td>3 (2–3)</td>
<td>1 (1-2)</td>
<td>2 (2-3)</td>
<td>2 (2-3)</td>
<td>2 (2-3)</td>
<td>2 (2)</td>
<td>2 (2)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Comorbidities (%)</td>
<td>17 (39%)</td>
<td>1 (1-3)</td>
<td>1 (1-3)</td>
<td>1 (1-3)</td>
<td>1 (1-3)</td>
<td>1 (1-3)</td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
</tbody>
</table>

An abnormal HLC-pair ratio was detected in 96% of MGUS and 94% of SMM patients, with no differences depending on the heavy chain isotype. A highly abnormal HLC ratio (<0.02 or >45) was present in 9 patients (1 with MGUS and 8 with SMM). HLC-pair suppression (i.e., IgG-x in patients with IgG-x gammapathy) was more frequent in patients with SMM (83% vs 46%, p=0.02). Severe HLC-pair suppression (>50% below lower level of normal) was present in 12 (27%) patients, the majority of which had a diagnosis of SMM (83%). Severe HLC-pair suppression was significantly associated with a highly abnormal (<0.125 or >8) serum free light chain (FLC) ratio (p=0.004), abnormal/normal bone marrow plasma cell ratio >0.95 (p<0.001) and immunoparesis (p=0.005), being present in 6 (86%) of the 7 patients with high risk SMM. Suppression of the other isotypes (i.e., IgA or IgM HLC pairs in a patient with IgG gammapathy) was identified in 33 (75%) patients, namely in 18 (69%) patients with MGUS and 15 (83%) patients with SMM(p=0.48), and was not significantly
associated with other risk factors for progression. Severe suppression (>50% below lower level of normal) was significantly more frequent in sEMD patients (33% vs 8%, p=0.04) and was associated with highly abnormal FLC ratio (p<0.001), abnormal/normal plasma cell ratio >0.95 (p<0.001), severe HLC-pair suppression (p<0.001) and highly abnormal HLC ratio at diagnosis (p<0.005). The "evolving" pattern of the serum M-protein was identified in 12 patients and 7, respectively. It was in significantly associated with either 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 isotope 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 is likely due to 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 1A). Our aim was studying the prognosis 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 EMM 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 performed only if the lesion was accessible (62%). 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 (parenchymal-EM) and bone (bEMD) were the most frequent sEMD patients has shown a poorer 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-plasmacytoma diagnosed accidentally after reconstructive breast-surgery, where Polymerase Chain Reaction of immunoglobulin decrease was performed in the clonal confirmed monoclonal-CD138/lambdla plasma-cells. This patient was at first treated with VTD-regimen followed by tandem-ASCT and after EM-relapse achieved complete remission with haploidentical-bone-marrow-transplantation. Allogeneic transplantation should however be remembered in the therapeutic-aramtamentarium against EM especially in high-risk-young patients. Furthermore often it has been described in the literature association between EMD, IgD subtype and FLC (free light chain)escape. In fact in our study we have reviewed 6 cases of IgD and 4 FLC-escape, all of them were observed in relapse-setting and in sEMD group. Finally the mechanism of extramedullary spread are poorly establislished due to low expression of integrins and CD56 is involved. In our population absence of CD56 protein was shown in 56% of sEMD group and in 15% of bEMD case-series.

Summary/Conclusions: Clinical features of MM-patients with bEMD were different from the patients with sEMD. Outcome of this population was significantly better than the patients with sEMD, and was comparable to the patients without EMD. Even in the era of novel drugs extramedullary soft tissue 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 difference 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=1.6-4.5; 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 (77% vs 60%; HR=2.9, 95%CI=1.3-6.8; p=0.001) and 5yr-OS (87% vs 69%; HR=2.9, 95%CI=1.5-5.6; p=0.001) was documented in patients receiving maintenance therapy, compared to those who did not receive maintenance therapy (48% vs 20%; HR=1.9, 95%CI=1.2-3.3; p=0.010) whereas initial therapy did not affect the outcome. Assigning a value to the variables found to be significantly related to survival measures, according to the above methods, patients were stratified into the following two groups: low-risk (LR), including 36 patients with score 0-1, i.e. patients achieving CR and receiving maintenance therapy (score 0) or achieving CR but not receiving maintenance (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-ISS II patients was significantly shorter than that of the LR group (20% vs 58%; HR=2.5, 95%CI=1.6-3.8; p<0.0001), whereas 5-year OS was 57% vs 80% (HR=1.9, 95%CI=1.1-3; p=0.021).

Summary/Conclusions: Our results suggest that in the R-ISS II MM patients,
the outcome of those achieving a CR and undergoing long-term therapy, is comparable with the outcome of the R-ISS I group. On the other hand, patients not achieving CR have a poor outcome, similar to those in the R-ISS III group. Therefore, these patients should require personalized therapy, aimed to achieve CR and to maintain therapy continuously.

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THE IMPACT OF THE UPDATED IMWG DIAGNOSTIC CRITERIA IN A REAL-LIFE SMM COHORT: A SINGLE CENTER EXPERIENCE

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Background: Recently, an update of the diagnostic criteria for smoldering multiple myeloma (SMM) & multiple myeloma (MM) was published by the International Myeloma Working Group (IMWG). In addition to CRAB criteria, 3 biomarkers of disease were introduced being (i) the presence of >60% clonal bone marrow plasma cells (BMP), (ii) a serum free light chain ratio (FLC) ratio >100 & (iii) the presence of >1 focal lesion on whole-body MRI (WBMRI). The introduction of these biomarkers has been 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 BMP and FLC ratio & biopsy were available. Analysis showed a significant higher estimate of BMP positivity was the most important factor. Although patients with SMM according to CRAB criteria were symptomatic and received MM therapy, also to confirm the VTE risk of IMIDs based-regimens and the relevance of anticoagulant thromboprophylaxis.

Methods: We retrospectively identified 28 SMM cases diagnosed between 01/01/09-31/12/14. Sufficient data for analysis was available for 25 patients. All patients underwent standard clinical & laboratory evaluation, bone marrow examination & WBMRI (T1- (+/-Gd) & T2-weighted sequences, diffusion-weighted sequences & additional DCEMRI sequences using time intensity curves). Time to progression (TTP) is defined as time from diagnosis until MM development. Overall survival (OS) is defined as time from diagnosis until death from any cause. Survival analysis was performed using the Kaplan-Meier method & significance was tested using the log-rank algorithm. Intergroup analysis was performed using non-parametric ranked-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 61.4 months (analysis performed on 01/02/2017). No patients had a FLC-ratio > 100 at time of diagnosis. Also, no patients with >60% of clonal BMPCs were seen. In 20 patients BMPC counts using both aspirate & biopsy were available. Analysis showed a significant higher estimate of BMP positivity was the most important factor. Although patients with SMM according to CRAB criteria were symptomatic and received MM therapy. Amongst the 164 patients that received IMIDs-based regimen, 27% did not receive any antithrombotic treatment, due to the lack of strong recommendations at the beginning of the use of IMIDs-based regimens. On the other hand, the median common thromboembolism was set with LMWH (54%), followed by low doses of aspirin (13%) and anti-vitamin K (VKA) (6%). Median follow was 40 months.

Conclusion: Based on our data, we support the actual recommendation of antithrombotic prophylaxis in IMIDs-based regimens, especially in association with high dose of dexamethasone. We recommend the use of a risk factor model including obesity and previous history of thromboembolic disease or cancer, in order to guide the appropriate thromboprophylaxis measures.

PB1957

A PHASE III RANDOMIZED, OPEN-LABEL STUDY OF ISATUXIMAB (SAR650984) PLUS POMALIDOMIDE AND DEXAMETHASONE VERSUS POM AND DEX IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Background: Treatment for refractory or relapsed and refractory multiple myeloma (MM) remains an unmet need. The most active agents in refractory MM are bortezomib, carfilzomib, and ixazomib. In patients who have received previous therapy, the combination of the limited activity of bortezomib/carfilzomib in these settings has prompted exploration of the clinical benefit of ISA in combination with Pom and low-dose dexamethasone (dex) (Pom/dex) versus Pom/dex for the treatment of adult patients with RRMM.

Methods: Eligible patients are those with RRMM and demonstrated disease progression within 60 days of the last therapy. Patients will have received at least 2 prior lines of therapy, including lenalidomide and a proteasome inhibitor (bortezomib, carfilzomib, or ixazomib) alone or in combination. Patients will be randomly assigned in a 1:1 ratio to either ISA (10 mg/kg IV on Days 1, 8, 15, and 22 in the 1st cycle; Days 1 and 15 in subsequent cycles) plus Pom (4 mg on Days 1–21) and dex (40 mg for patients <75 years of age and at 20 mg for patients ≥75 years of age) or Pom (4 mg on Days 1–21) and dex (40 mg for patients <75 years of age and at 20 mg for patients ≥75 years of age) and 40 mg dexamethasone. Treatment cycles will be 28 days each. Patients will continue therapy until progression, occurrence of unacceptable adverse events (AEs), or their decision to discontinue the study, whichever comes first. All patients will be required to provide informed consent. The primary endpoint is progression-free survival (PFS), i.e. time from randomization to progressive disease or death from any cause. Response will be determined by IMWG criteria (2016). Key secondary endpoints include overall response rate and overall survival (OS). Safety evaluations include treatment-emergent AEs/serious AEs (including infusion-associated reactions), laboratory parameters, vital signs, and assessment of physical examination

Results: Approximately 300 patients (150 in each arm) are expected to be enrolled in this study. Statistical analyses will be conducted according to a pre-specified plan. The first patient was recruited in January 2017.

Summary/Conclusions: This Phase III, prospective, multicenter trial will provide new data from randomized comparison of the efficacy and safety of different treatment regimens for RRMM, a combination which has previously reported preliminary clinical activity and manageable toxicities in heavily pretreated patients with RRMM in a single-arm Phase Ib study.
LONG TERM SURVIVAL OF IGM MULTIPLE MYELOMA AND WALDENSTRÖM’S MACROGLOBULINEMIA PATIENTS

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Background: IgM multiple myeloma (MM) and Waldenström’s macroglobulinaemia (WM) are two hematologic malignancies with the common finding of monoclonal gammopathy. IgM MM is a rare and poorly characterized disease.

Aims: The paper presents clinical and laboratory results of long term observations of 15 IgM MM patients selected from a group of 889 MM patients (16%) diagnosed and treated for several years at the Institute of Hematology and Transfusion Medicine in Warsaw as well as 15 WM patients investigated and treated at the same period of time at our hospital.

Methods: For analysis of serum proteins new Hevylite and Freelite tests (Binding Site Ltd, Birmingham, UK) were applied as well as immunofixation using antibodies against kappa, lambda, and free light chains. Fresh and archived frozen serum samples were used for the study.

Results: The clinical presentation of IgM MM patients is heterogenic starting with typical form for non IgM MM through predominant form with characteristic hyperviscosity syndrome and severe disease course to slow and latent form with survival time up to dozens of years. In 2 patients diagnosis of IgM MM was preceded by a 3-year period of monoclonal gammopathy of undetermined significance (MGUS) while in 4 patients (27%) diagnosis of WM was preceded by a 108, 84, 78, 9 months period of IgM MGUS. Mean real overall survival of IgM MM patients was 50 months, 5 patients (33%) survived above 7 years and 13 patients (86%) survived above 12 years. Mean survival of WM patients was 108 months, 7 patients (47%) survived above 10 years, 3 patients (20%) survived above 15 years. Lytic bone lesions were found in 11 (73%) IgM MM patients and in 3 (20%) WM patients. Urine monoclonal light chains (FLC) detected by immunofixation was present in 60% of IgM MM patients and in 5 (20%) WM patients. Urine monoclonal light chains (FLC) ratio in serum (by Freelite) in 75% of IgM MM patients. It was shown that IgM clonality in IgM MM and WM patients can be determined by using immunoglobulin heavy chain/light chain (HLC) immunoaassays- Hevylite. Immunofixation and HLC ratios were concordant in all assessed IgM MM and WM patients. In IgM MM patients 133% of uninvolved polyclonal IgM and IgG were detectable using immuno 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 cell gM was 15 months and in patients with normal polyclonal IgM 55 months (p<0.01).

Summary/Conclusions: 33% of IgM MM patients survive above 7 years and 13% above 12 years while 47% of WM patients survive above 10 years and 20% above 15 years. Suppression of uninvolved polyclonal IgM (detectable by using HLC test) at the time of IgM myeloma diagnosis is unfavorable prognostic factor.

MULTISITE PERFORMANCE EVALUATION STUDY OF THE BD ONEOFLOW 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) Consorquium and reagent system. The BD OneFlow solution enables reproducible identification and discrimination of distinct cell populations by combining stan- dardized assays, setup reagents, and protocols. The plasma cell disorders (PCD) panel is composed of the BD OneFlow PCST (Plasma Cell Screening Tube) and BD OneFlow PCD. BD OneFlow PCST helps differentiate normal plasma cell populations from those requiring follow-up. The BD OneFlow PCD classification tube helps differentiate abnormal from normal plasma cell populations. The BD OneFlow PCD tube, when run in parallel with BD OneFlow PCST tube, characterizes the abnormal plasma cell population for identification of plasma cell disorders.

Aims: The objective of this study was to compare the accuracy between the BD OneFlow PCD system and the EF liquid comparator system.

Methods: De-identified remnant human bone marrow specimens (n=48) were collected at two study sites and tested in an blinded manner within 24 hours of draw. Specimens were simultaneously stained with BD OneFlow PCD and BD OneFlow EF using the EF specific protocol. A total of 22 samples were analyzed 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. 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 PCST and PCD tubes and the EF liquid reagent system were 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 method for aiding in the diagnosis of plasma cell disorders from bone marrow specimens.

PB1960

PRACTICE GAPS AND BARRIERS TO OPTIMAL MANAGEMENT OF MULTIPLE MYELOMA PATIENTS: RESULTS FROM A MIXED-METHODS STUDY IN 8 EUROPEAN COUNTRIES

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Background: Previous studies have identified gaps and barriers in Multiple Myeloma (MM) patient care, especially in relation to treatment decision making. However, there is a need for better understanding the practice gaps, from the healthcare providers’ perspectives, with the purpose to investigate the root causes of those gaps and find solutions to alleviate the challenges.

Aims: We conducted a study to identify the practice gaps and challenges in the diagnosis, treatment and management of MM patients, as experienced and reported by medical oncologists, hematologists and hemato-oncologists (HEM) and oncology nurses (NU) in 8 European countries between February 2016 and June 2016.

Methods: This mixed methods ethics-approved study included exploratory semi-structured interviews (phase 1) designed to generate in-depth discussion about unmet needs and challenges in the diagnosis, treatment and management of MM, followed by a quantitative online survey (phase 2) designed to validate the findings from the interviews with a larger sample. Practice gaps were identified through combined analysis of data from the in-depth interviews and online surveys.

Results: A total of 364 participants (HEM=281, NU=83) from France (n=58), Germany (n=58), Russia (n=25), Italy (n=26), the Netherlands (n=16), and Belgium (n=25) participated in this study. Thirty-nine (39) interviews were conducted (HEM=28, NU=11) and 325 participants completed the online survey (HEM=253, NU=72). A majority (79%) of the sample had more than 10 years of clinical practice experience and over a third (39%) had over 20% of MM patients in their patient caseload. Three key finding were identified in the management of MM patients: 1) challenges in managing treatment side-effects. Forty percent (40%) of HEM reported lack of skills in managing cardiovascular side effects or symptoms. Over a third of HEM reported difficulties in managing fatigue (40%), skin toxicities (35%) or peripheral neuropathy (34%). NU reported more barriers (46%) with peripheral neuropathy (36%) as a side effect or symptom (46%), peripheral neuropathy (36%), thrombosis (37%), and skin toxicities (33%). Additionally, 2) NU reported challenges in communicating with patients and educating them around their disease, especially in relation to treatment outcomes and long term side effects. For example, 51% of NU reported a lack of skills discussing sexual issues as consequences of the disease/treatment. Finally 3) there was a large variability across countries in the guidelines followed by HEM and NU for the treatment and management of MM patients. Detailed results, including country-specific analyses and investigation of the practice gaps’ causalities, will be presented.

Summary/Conclusions: These findings provide real-life recent evidence of the challenges of HEM and NU in relation to specific aspects of the manage- ment of patients with MM with 3 main areas, challenges in managing side effects, communication with patients and leverage of guidelines which show differences between HEM and NU but also between countries. The findings of these mixed methods study can help inform healthcare providers of educational activities and performance improvement interventions, adapted to the local context at a country level. Efforts should aim to address those current challenges before new therapies, such as immunotherapies, become available.
The expression of APRIL by multiple myeloma cells and their role in the evolution of multiple myeloma.

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. Induced factors secreted by tumor cells and other cells of the marrow microenvironment play an important role in disease progression. APRIL, by initial letters A Proliferation Inducing Ligand, is a member of the pro TNF, one of the main factors for the survival of immature and activated B cells. One of the main signal transduction pathways for activation of myeloma cells is NF-κB. APRIL, can directly activate the NF-κB and has been found by studies that are the most important factors for the survival of healthy and myeloma cells.

Aims: Aim of this study was the study of APRIL expression in myeloma cells in the bone marrow of patients with MM and their possible association with cell proliferation and malignancy.

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, 1 stage II and 1 stage III. Regarding the type of paraprotein that had been found, 23 patients had IgG, 14 IGA and 5 patients with light chains. 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. 20 age and sex-matched healthy volunteers, were used as controls. The levels of IL-10 and IL-6 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 evasculated as the percentage of neoplastic plasma cells and according to the intensity of staining in four-grade scale 0: negative, +1 weak, +2 moderate and +3 intense staining. Then the proportion of plasma cells stained for each type of staining separately, was calculated using the H-score method (Histoscore), based on the formula: % *% * 2+3% . Our aim is to prove if the intensity of expression is associated with disease stage.

Results: Statistically significant differences were observed between patients and controls for all parameters measured (p<0.001 in all cases) . All values of the measured parameters increased in parallel with the ISS stages of the disease. p<0.001 for 0.0% Ki-67 infiltration, p<0.01, IL-10 <0.001, IL-6, p<0.001) Eventually APRIL correlated significantly with all measured parameters e.g. BM infiltration r=0,386, p<0,01, with Ki-67 r=0,390 p<0.01 IL-10 r=0,497 p<0.001, IL-6, r=0,484 p<0.001.

Summary/Conclusions: Increased expression of APRIL ligand plays an important role in development and pathology of MM and may be an important therapeutic target in the treatment of MM.
(45%), and local discomfort for EMP. 52% of patients presented a MB at diagnosis, without significant differences between subgroups. With 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.

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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 treat-ment (2L), and previous treatment outcomes to stratify patients based on their overall survival (OS) expectations from initiation of 2L treatment (Hajek et al. Blood 2016). The value of such an algorithm depends on its validation, but also on understanding the evidence that explains these differences in survival expectations.

Aims: To describe 2L treatment patterns by RSA group and to report OS, pro-gression-free survival (PFS) and response by treatment received in 2L per RSA risk group.

Methods: Data were collected from the Czech RMG for patients aged ≥18 years who were diagnosed with symptomatic MM between May 2007 and April 2016 and in whom 2L treatment had been initiated. Predictors of OS from the start of 2L were identified using Cox regression analyses. Hazard ratios for each OS predictor were multiplied to obtain an overall score for each patient. Risk groups were defined based on the overall score. To provide optimal patient stratification, cut-offs of the score were estimated using K-adaptive partitioning for survival (KAPS) analysis.

Results: Data from 1418 patients were analysed. KAPS analysis defined four groups based on risk of death: low (LR; score ≤ 4.1; n=403), intermediate-low (ILR; score 4.2–10.3; n=635), intermediate-high (IH; 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, IH 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 lenalido-mide 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 sug-gests that 2L treatment choice was not defined by the underlying risk of death for each patient, but rather by the type of previous treatment. For patients receiving lenalidomide at 2L (months from start of 2L was 57, 29, 13 and 6 (Figure 1), and median PFS (months) was 18, 12, 8 and 3 in the LR, ILR, IH and HR groups, respectively. A very good partial response or better (VGPR+) was reported for 29.3%, 31.0%, 18.7% and 16.8% of patients in the LR, ILR, IH 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, IH and HR groups, respectively. A VGPR+ was reported for 33.6%, 22.9%, 26.0% and 7.1% of patients in the LR, ILR, IH 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.

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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 accumu-lation 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 infil-tration and peripheral blood involvement, higher incidence of extramedullary disease, renal insufficiency, Bence Jones protein, plasma cell leukemia and t(11;14). The lack of CD117 expression is associated with higher levels of bone marrow infiltration, renal impairment, elevated β2-microglobulin and cytogenetic...
aberrations including t(11;14), t(4;14) and del(13q). CD28 expression is present in 15–45% of patients and is associated with unfavorably cytogenetic changes including t(4;14) and del(17p) and shorter PFS and OS despite aHSCCT. Aims: Aim of our retrospective study was to evaluate the impact of CD56, CD117 and CD28 expression on clinical characteristics and PFS in newly diagnosed MM patients treated with bortezomib based induction therapy. Methods: We retrospectively analyzed 110 newly diagnosed MM patients from our national registry that had data available at the time of diagnosis. Immunophenotype was determined using a panel consisting of CD19/CD38/CD45/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, elevated LDH or RISS stage. We found a strong association between lack of CD56 expression and light-chain only or asymptomatic myeloma. There was an association between CD28 expression and female gender (Table 1). In multivariate analysis including age, elevated creatinine, RISS, aHSCCT, 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 aHSCCT and age. In patients not undergoing aHSCCT 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.

Table 1

Summary/Conclusions: CD56 expression was prognostic for PFS only in the patient cohort not undergoing aHSCCT. As previously reported aHSCCT seems to abrogate the negative impact of CD56 negativity. We propose CD56 expression to be used as a prognostic marker in patients with RISS stage 2 disease and to be used possible these patients should undergo aHSCCT.

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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. The primary objective is to describe the overall use of ASCT in routine and by online personal checks. We investigated which institutions initiated treatment in patients with ASCT, which were the characteristics for patients not-considered eligible for transplantation, how stem cell mobilization was performed, how many patients dropped out before planned transplantation and what were the frequencies of tandem ASCT and ASCT for relapsed disease. Results: Data from 515 patients from 51 centres were available for the first half of 2015 and from 867 patients from 52 centres for the first half of 2016. There were 40% (2015) and 32% (2016) pts considered as eligible for ASCT in 1st line. Although the proportion of patients older than 69 years was not significantly different between health care providers in 2015 and 2016 (2015: 47%/UH, 60%/CH, 49%/OBH; 2016: 54%/UH, 56%/CH, 47%/OBH), patients were considered more often transplant-eligible in UH (2015: 49%/UH, 36%/CH, 45%/OBH) than in CH (2015: 39%/UH, 26%/CH, 21%/OBH). In first-line treatment, 52% of patients eligible for SCT received mobilization chemotherapy in addition to induction therapy. More than 80% of these patients received a cyclophosphamide-based chemotherapy in combination with G-CSF for stem cell mobilization in 2015 and 2016. Most participating institutions aimed at collection of three sufficient stem cell transplants. In 2015 (43% of patients received ASCT), patients 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%). 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 (RMM). 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%/76%) or immunomodulatory agents (82%/68%) 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. Results: We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RMM). 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%/76%) or immunomodulatory agents (82%/68%) 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. Results: We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RMM). 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%/76%) or immunomodulatory agents (82%/68%) respectively.

Summary/Conclusions: CD56 expression was prognostic for PFS only in the patient cohort not undergoing aHSCCT. As previously reported aHSCCT seems to abrogate the negative impact of CD56 negativity. We propose CD56 expression to be used as a prognostic marker in patients with RISS stage 2 disease and to be used possible these patients should undergo aHSCCT.

PB1967

MODIFIED HYPERCVAD/ERUS 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).

Aims: The primary objective is to describe the safety and efficacy of the hyperCVAD 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 (RMM). 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%/76%) or immunomodulatory agents (82%/68%) 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. Results: We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RMM). 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%/76%) or immunomodulatory agents (82%/68%) 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. Results: We describe the effectiveness and tolerability of the 2 regimens among 33 patients with relapsed and/or refractory multiple myeloma (RMM). 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%/76%) or immunomodulatory agents (82%/68%) respectively.
PB1968

EFFICACY AND SAFETY OF LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: A REAL LIFE EXPERIENCE FROM TURKEY

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Background: Lenalidomide, an immunomodulatory drug, was approved for treatment of relapse/refractory multiple myeloma (RR-MM). In Turkey, we have been used the combination of lenalidomide and dexamethasone (RD) for RR-MM patients after 2010. Therefore, we analyzed efficacy and safety of RD in Turkish patients with RR-MM.

Aims: We aimed to evaluate the outcome and the tolerability of the RD in patients with RR-MM who had been treated under the standard clinical practice between October 2010 and June 2016.

Methods: This is a retrospective, single center study. Patients’ clinical and laboratory data were collected from patient files. The overall and progression free survival (OS and PFS) were measured. OS and PFS were measured from the start of the RD. The median number of previous line of treatment was 1 (4–7). 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 (25 mg) per day for 21 days in a cycle of 28 days. Objective response rate (ORR) was observed in 87 patients (72.5%); 23 patients (19.2%) achieved CR. The median follow-up was 14 months (range: 1–72 months), and the median DOR was 19 months (range: 12.4–25.6 months). Median OS and PFS were 32 months (95% CI, 15.8–48.1 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 discontinue rate due to AEs was 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 herpetic 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 seems 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


Background: During many years, the combination of lenalidomide and dexamethasone (RD) has been an effective treatment for patients with relapsed or 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 evaluated efficacy and safety of RD in Turkish patients with RR-MM.

Methods: We retrospectively reviewed a consecutive cohort of patients with RR-MM 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 thrombopoietrophylaxis with low-molecular-weight-heparin (LMWH) (Enoxaparin 40 mg subcutaneous daily) the first 4 cycles; thereafter, patients were switched to aspirin 100 mg in a day prophylaxis. Bemiparin 7500 anti-Xa IU once-daily dose was employed if enoxaparin was suspended. Clonazepam dose to treat dystonia was 0.5 mg twice daily. Data were analyzed with SPSS statistical v 22.0.

Results: Between 2011 and 2016 a total of 65 patients received Rd in our center. Baseline characteristics are shown in Table 1. Patients received a median of 2 previous regimens (range 1-6). 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 esteroids or antihistaminic were administrated. Dystonias were reported in 23.1% of patients (grade 2), all of them disappeared after treatment with clonazepam without lenalidomide dose reduction.

Table 1.

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

E. Salido Fierrez, J. M. Moraleda Jimenez

Aims: To explore: I) the incidence of late thrombotic events in a real world population of relapsed MM, addressing to Len-dex 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 86%, CR 7%. Table 1 shows type and distribution of risk factors for VTE. In details median number of VTE risk factors per patient was 2 (range 0-6), 58.2% of pts had ≥2 risk factors, 41.8% of pts had 0-1 risk factor for VTE. Median duration of TP is 4.8 months (range 0-12 months).
0.4-6 months). No hemorrhagic events were observed during LMWH. Cumulative incidence of VTE was 11.7% (12/103 pts), similar to that previously reported in the literature in patients with continuous TP. The median time from nelalidomide starting and VTE occurrence was of 12.2 months (range 1- 88.2 months), with only one patient developing early VTE among our group. In detail we observed 10 deep vein thrombosis (83%), 1 pulmonary embolism (8.5%), 1 myocardial infarction (8.5%). Most of patients developing VTE had good disease control (≥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>% of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;65 years</td>
<td>56</td>
<td>61%</td>
</tr>
<tr>
<td>CVC/Port</td>
<td>52</td>
<td>57%</td>
</tr>
<tr>
<td>Vascular catheter</td>
<td>22</td>
<td>24%</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>52</td>
<td>57%</td>
</tr>
<tr>
<td>PS 1</td>
<td>66</td>
<td>71%</td>
</tr>
<tr>
<td>PS 2</td>
<td>28</td>
<td>30%</td>
</tr>
<tr>
<td>PS 3</td>
<td>10</td>
<td>11%</td>
</tr>
</tbody>
</table>

Summary/Conclusions: This study shows that LMWH is effective and well tolerated for early VTE prophylaxis during Lenalidomide plus low dose Dexamethasone. Incidence of late VTE without TP maintenance is similar to that reported with long-term 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 reported results of clinical trial.

Aims: We examined the clinical parameter to assess survival in elderly patients with newly diagnosed MM (NDMM) in the real world is different from the results of clinical trial.

Methods: We performed a retrospective study involving 125 elderly NDMM patients from April 2012 to September 2015. Patients aged 60 years or older, who were ineligible for autologous stem cell transplantation, were selected. The study included 57 males and 68 females, with median age at diagnosis of 74 years (range, 60-95 years). ECOG performance status at diagnosis were 0-1, 67; 2-4, 38. We collected pretreatment parameter at diagnosis as follows; monoclonal protein type (IgG 60; IgA 32; IgD 1; B/JP 30; non-secretory 2), light chain (kappa 72; lambda 52; unknown), hemoglobin level (mean 8.9 g/dL [range 5.8-15.2]), calcium level (mean 10.0 mg/dL [range 8.7-20.2]), estimated glomerular filtration rate (eGFR) (mean 93.3 mL/min [range 56.0-114.2]), 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 [range 83-213]), cytogenetic abnormalities by using fluorescence in situ hybridization (FISH) [none, 53; t(4;14), 7; del(17p), 14; t(4;14) & del(17p), 5; t(4;14) & t(14;16) & del(17p), 1].

Results: Of 125 patients, 76 patients received bortezomib based therapy (VMP, 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, 62; N/A, 4, can divide elderly patients into three distinct survival groups (P<0.001) (Figure 1A).

Univariate and multivariate analysis showed a lower OS was associated with serum calcium level greater than 11 mg/dL (HR 3.036, 95%CI 1.412-6.529) (Figure 1C). Among 80 patients with FISH data, survival of those with t(14;16) or del(17p) or t(14;16) was not statistically different (P=0.394).

Survival of patients treated with bortezomib or lenalidomide as an induction therapy was better, while not statistically significant (P=0.066) than those who were not.

Figure 1.

Summary/Conclusions: This study shows that LMWH is effective and well tolerated for early VTE prophylaxis during Lenalidomide plus low dose Dexamethasone. Incidence of late VTE without TP maintenance is similar to that reported with long-term antiplatelet therapy. We found no difference in factors predisposing for thrombosis among patients developing or not VTE, with a not negligible proportion of concomitant adverse events observed nearby VTE occurrence.
even both. First line treatment 56% of the patients received Bortezomib based triple therapy. 22% received CTD (Cyclophosphamide, Thalidomide, Dexamethasone), 7% RD (Lenalidomide, Dexamethasone), 3% CyBord (Cyclophosphamide, Bortezomib, Dexamethasone), 3% RV (Lenalidomide, Bortezomib), 2% Thal-Dex (Thalidomide, Dexamethasone), 2% RT (Radiotherapy), 2% WatchfulWait, 1% MP (Melphalan, Prednisone) and 3% refused for treatment and lost follow up.

Table 1.

<table>
<thead>
<tr>
<th>ISS stage</th>
<th>% of patients</th>
<th>ISS stage</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>24</td>
<td>Stage II</td>
<td>31</td>
</tr>
<tr>
<td>Stage II</td>
<td>31</td>
<td>Stage III</td>
<td>47</td>
</tr>
<tr>
<td>Stage III</td>
<td>47</td>
<td></td>
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</tbody>
</table>

Summary/Conclusions: Applying the RISS system to myeloma patients is a very effective and easy method to categorise myeloma patients, a significant number of patients in Kuwait are diagnosed as stage III, with median age of 56 years although the use of novel therapies shows excellent response to most of them.

PB1974

EPIDEMIOLOGY OF MULTIPLE MYELOMA. THE GRANADA MYELOMA REGISTRY

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Background: The Granada Myeloma Registry is the second largest single-institution population-based registry (Ríos-Tamayo et al, 2015) of multiple myeloma (MM) referenced to date. Here we update and point out the 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 (solitary plasmacytoma, monoclonal gammopathy of undetermined significance, or smoldering MM), and 14 patients (2%) remain alive with smoldering MM without progression. The subtype of MM is IgG 55.6%, IgA 24.8%, Light Chain Only 4%, Non-secretory 15.9%, Non-secretory 3%, IgD 0.6% and IgM 0.2%. The International Staging System (ISS) is used in 378 patients (98.9%). 2 (25.7%) and 3 (41.4%) patients are ISS stage III, and lost follow up.

PB1973

FEASIBILITY/PHASE II STUDY OF MYELOABLATIVE BEAM ALLOGENEIC PB1973 TRANSPLANTATION FOLLOWED BY ORAL IXAZOMIB MAINTENANCE THERAPY IN PATIENTS WITH HIGH RISK MYELOMA

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Background: While the role of allo-HCT in MM remains controversial several studies have shown encouraging PFS and OS with this treatment even in patients with high-risk myeloma (HRM). HRM manifests with early relapses and refractoriness. Median OS is 2.5 years despite aggressive therapy with novel agents. Post auto-HCT maintenance with lenalidomide is considered standard of care, but post allo-HCT maintenance presents unique challenges and has not been well studied. Ixazomib (Ixa) is a new oral proteasome inhibitor with demonstrated activity in patients with refractory MM, For patients with high-risk MM (HRM) who have failed autologous transplantation and relapsed with refractoriness. Median OS is 2.5 years despite aggressive therapy with novel agents. Post auto-HCT maintenance with lenalidomide is considered 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.

Methods: The protocol was approved by a local institutional review board and ethics committee. The study was conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent prior to treatment. Eligibility criteria include: age ≤65; relapsed MM previously treated with auto-HCT. The BEAM conditioning regimen includes: BCNU 200 mg/m² on day -29; carmustine 400 mg/m² mg twice daily -5 to -2; etoposide 200 mg/m² mg twice daily -5 to -2; etoposide 200 mg/m² mg twice daily -5 to -2; melphalan 140 mg/m² on day -1. Oral Ixa 4mg on days 1 and 14 of a 28-day cycle. Ixa may start between day -1 and 4 PR. Treatment continues for 28 cycles (1 cycle every 28 days for up to 2 years) and is continued for up to 24 cycles.

Results: Six subjects were enrolled, 3 at OHSU and 3 at Duke, from Sept 2015 to Dec 2016. Median age of 51 (range 46-57), 2 female, and all of white race. High risk factors: del(17p), t(4;14), t(14;16), t(14;20), amp1q gain or del1p, del13q+ and lost follow up.

Summary/Conclusions: While this is very early data, it is the first clinical trial to report the use of BEAM conditioning followed by Ixa maintenance for relapsed HRM. Thus far stopping rules have not been met, with expected toxicities occurring.

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REAL WORLD USE OF IXAZOMIB WITH LENALIDOMIDE AND DEXAMETHASONE FOR PATIENTS WITH RELAPSED AND RELAPSED REFRACTORY MULTIPLE MYELOMA

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Background: Ixazomib (Ixa) is a novel oral proteasome inhibitor (PI) approved in combination with lenalidomide and dexamethasone (IRD) for the treatment of relapsed/refractory multiple myeloma (MM). This was based on the TOUR-MALINE-MM1 trial which demonstrated a progression free survival benefit over RD. However real world use often differs to clinical trials due to heterogeneous patient selection, more flexibility with dosing intensity and country specific prescribings practices/funding restrictions.

Aims: To characterise real world use of IRD by demographics, response rate (RR) and progression free survival.

Methods: This was a retrospective review of patients sequentially treated with IRD at a large UK Haematology Centre. Patients received Ixa 4mg D1, 8, 15 with lenalidomide (dose as per label) days 1-21 and dexamethasone 40mg weekly or as tolerated every 29 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.

Aims: To characterise real world use of IRD by demographics, response rate (RR) and progression free survival.

Methods: This was a retrospective review of patients sequentially treated with IRD at a large UK Haematology Centre. Patients received Ixa 4mg D1, 8, 15 with lenalidomide (dose as per label) days 1-21 and dexamethasone 40mg weekly or as tolerated every 29 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 12 (40%), 27% had received 2 or more 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 progres-

Summary/Conclusions: 8.5% of patients were refractory to the local chemotherapy. The median PFS in the local cohort 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 experiencing grade 3 or 4 neutropenia, thrombocytopenia and 1 patient experiencing grade 4 anaemia. This resulted in ixa dose reductions in 4 (16.7%) patients. Ixa was stopped in 1 patient due to adverse events.

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Background: New agents have revolutionised the treatment of multiple mye-

PB1976

EFFECTIVITY AND TOLERABILITY OF LENALIDOMIDE AND POMALIDOMIDE IN RELAPSED/REFRACTORY MYELOMA PATIENTS IN A REAL WORLD STUDY

Aims: In our study we aimed to describe the real-world experience of the use of lenalidomide followed by pomalidomide rescue in a relatively elderly co-mor-

Methods: Records of delivered chemotherapy cycles were retrieved from local pharmacy data and national averages from Celgene ePAF data. Outcome data collected from clinical notes and laboratory results.

Results: We collected data on 46 patients treated between 2011-2014 with lenalidomide, 17 whom progressed to receive pomalidomide. The median age at initial diagnosis was 71 years, with median age at starting lenalidomide 77 years (range 36-94). This gave an average of 5 years from diagnosis to commencing lenalidomide (range 1-15 years). Myeloma subtypes included IgG 28/4, IgA 11/46, light chain disease 4/46 and 3 with IgD and non-secretory myeloma. High risk cytogenetics [17p-, (4;16), t(4;20), hyperdiploidy, chromosome 1 abnormal-

Background: Multiple myeloma (MM) is one of the most prevalent hematopoietic malignancies in the world. The 2014 revised International Myeloma Working Group (IMWG) guidelines for MM treatment recommend lenalidomide as initial treatment for patients with advanced smoldering myeloma, not eligible for ASCT, and for patients who have received prior treatment with lenalidomide or bortezomib. However, IMiD agents can be associated with adverse events, drug-related dose reductions, and dose interruptions. Aims: This study aimed to assess the effectiveness and tolerability of lenalidomide and pomalidomide as initial treatment in a real-world scenario.

Summary/Conclusions: We conclude from this real-world retrospective review of 2nd and 3rd line IMiD therapy that these salvage regimens are highly effective. Patients on lenalidomide monotherapy post triplet/duplet induction were often re-escalated back onto dexamethasone and alkylator (IV/oral) based regimens with successful salvage, contributing to the observed long duration of local therapy compared to national averages. Pomalidomide was highly effective at rescuing patients failing lenalidomide-based regimes and well tolerated.

PB1977

APPLICATION OF CONDITIONING REGIMEN WITH BUSULFAN AND CYPHOSPHAMIDE IN AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA

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Background: Busulfan is the most commonly used drug in conditioning regimens for hematopoietic stem cell transplantation and high-dose melphalan (MEL) is the standard conditioning regimen in autologous stem cell transplantation. The median time to neutrophil engraftment in the BUS and MEL groups were 10(8-17) days versus 10(9-13) days, taking the same time on average (P=0.46). 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 SR/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 MEL group, the median follow-up was 12.5 (0-26) months. Six patients (16.7%) underwent disease progression. The 2-year progression-free survival (PFS) rate was 68%. Correspondingly, in the BUS 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 BUS regimen is a safe and effective therapy for ASCT in patients with multiple myeloma. Besides, BUS regimen is not inferior to MEL regimen. In conclusion, BUS regimen may replace MEL 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. 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 BUSY regimen while the others received high dose mel-

Results: Of the 12 patients with MM and CNS involvement treated in our centers, 9 were males and 3 were females. The median age was 58 years (range 29-83). The median time on treatment currently was 5.5 months (5.6-40). The median number of treatment cycles was 4 (1-10). The median PFS in the local cohort was 53.5 months. The average number of cycles in those who progressed to pomalidomide was 12.8 (n=17), which is double that of the national average reported in seminal trials. These patients had few treatment breaks and treatment was well tolerated (pomalidomide duplets or triplets).

Summary/Conclusions: We conclude from this real-world retrospective review of 2nd and 3rd line IMiD therapy that these salvage regimens are highly effective. Patients on lenalidomide monotherapy post triplet/duplet induction were often re-escalated back onto dexamethasone and alkylator (IV/oral) based regimens with successful salvage, contributing to the observed long duration of local therapy compared to national averages. Pomalidomide was highly effective at rescuing patients failing lenalidomide-based regimes and well tolerated.

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progression was 23.9 (3-65) months. Eight patients presented with cerebral nerve palsies, 2 with paraplegia, 1 with hemiparesis and 1 with headache. CSF cytospin or flow cytometry was positive in 7, MRI or CT supported the diagnosis in 4 patients. Treatment consisted of combination chemotherapy, intrathecal chemotherapy, cranio-caudal radiotherapy and imids with various success. The PFS and OS from CNS progression was 63 and 125 days. Two patients survived for over a year (427 and 778 days), both responded in terms of CNS symptoms to imid-based combination therapy and one had cranio-caudal radiotherapy (Figure 1).

Figure 1.

Summary/Conclusions: CNS progression in MM has a particularly poor prognosis as it represents a late stage of an aggressive relapse which often shows chemo-refractoriness. The differential diagnosis includes infection, autoimmune or vascular diseases of the CNS as well as paraneoplasia and drug toxicity. The CNS penetration of the effective myeloma drugs is poor except for the imids, and drugs with CNS availability are usually not very effective in refractory MM.

PB1979

DARATUMUMAB: CHALLENGES OF INTEGRATING THIS NEW THERAPY INTO STANDARD CARE

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Background: Daratumumab (Darzalex) is the first anti-CD38 human Monoclonal Antibody approved for Multiple Myeloma (MM). Targeting the CD38 antigen on the surface of MM cells it causes apoptosis, and has an immune modulated tumour lysis effect. Success in Clinical trials meant that this drug, administered as single agent, or in combination with other novel therapies (Lenalidomide or Bortezomib), received accelerated FDA Approval in the US. It is now being introduced into standard hospital care.

Aims: Daratumumab presents unique challenges to the delivery of risk managed care, due to effects on some blood and bone marrow testing, and to the Infusion Related Reactions (IRRs) seen at the outset of treatment. This poster will highlight important aspects of the treatment pathway for this new therapy, from a single centre perspective.

Methods: We outline the pathways integrated at MDT level; patient characteristics and adverse event profiles of the 15 myeloma patients we have treated with Daratumumab, in a standard service setting.

Results: Daratumumab affects certain pathology tests so samples should be clearly identified. Relevant laboratory teams need to be aware of the methods used to process samples. Daratumumab binds to CD38 on Red Blood Cells, and therefore with Cross Match Compatibility testing and Antibody Screening. Obtaining RBC Products for patients receiving Dara will take longer, requiring up to 48 hours notice. Cross match samples taken prior to treatment provide the National Blood Service Laboratory with a baseline antigen profile to aid selection of suitable blood products. Dara is detected during Paraprotein Electrophoresis; Pre and Post baseline samples help to identify the Darzalex Band in the serum; our lab use a Sebia capillary 2 analyser to separate the Dara band for accurate reporting. Bone Marrow Testing: Daratumumab affects Immunophenotyping by masking the CD38 epitope used to identify plasma cells by flow cytometry; special kits are available using a different CD38 epitope thus dealing with this issue. Infusion Related Reactions (IRRs) have been reported in over half of patients receiving Daratumumab: 96% of these were seen at the first dose. Typically involving the upper respiratory tract and include rhinitis, cough, wheeze, bronchospasm, laryngospasm and chest pain. More rarely they include rash, fever, and nausea. Reactions can be grade 1-4 so it’s important that the patient is closely monitored where there is quick access to specialist staff, resuscitation equipment and respiratory support in a high dependency setting. Staff training is important and patients need 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>Gender</th>
<th>Number of prior treatments</th>
<th>Regimens</th>
<th>Disease outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: 8</td>
<td>2-14</td>
<td>Single agent alone</td>
<td>Response: 14 (78% complete)</td>
</tr>
<tr>
<td>Female: 7</td>
<td></td>
<td>Daratumumab with Lenalidomide</td>
<td>7 (50%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Daratumumab with Bortezomib</td>
<td>8 (53%)</td>
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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 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 patients on HAART was lower compared to that of HIV-negative MM patients. The survival of the HIV+ MM patients were also superior to that of non-HIV MM patients reported in the literature. The majority of HIV+ MM patients who had long-term follow-up in our study did not show clinical symptoms of MM and were free of serum-M protein after primary MM therapy in the presence or absence of HAART and maintained treatment with HAART alone. Although MM is not an AIDS-defining illness, meta-analyses of large population studies reveal an increased risk of MM in HIV/AIDS patients. HIV infection is commonly associated with B cell hyper-proliferation, 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 ossalgia and inability to move. Total protein 135 g/l with 81.7 g/l of IgG-k M-protein and no presence of Bence Jones protein. Bone skeletal survey showed multiple generalized lytic lesions. Bone marrow aspirate and biopsy showed 46% plasma cells. Serum creatinine – 104 mkmol/l. HIV and hepatitis C (genotype 1a) screening test was positive, confirmed with Western blot analysis. The CD4 count was 290 cells, HIV viral load 1500 copies/ml, hepatitis C viral load 14.2 mln copies. He was started on HAART, combined with chemotherapy 5 courses of CP+CVP+MP and 7 V-MP. In 2017 total serum protein 97.3 g/l, M-protein 31.2 g/l, serum creatinine 63.0 mkmol/l. Now he is active without any bone pain receives Pegasys and lamivudine (Table 1).
Background: Autologous stem cell transplant (ASCT) is a well-established treatment for myeloma. However, the optimal strategy for stem cell mobilization remains undefined. The goal of mobilization is to collect adequate stem cells for at least 2 ASCT (4x10^6/kg), with the minimum apheresis sessions and toxicities such as febrile neutropenia.

Aims: We aim to compare stem cell mobilization using granulocyte colony stem cell factor (GCSF) only (steady state), high dose cyclophosphamide (4 g/m2) with GCSF or low dose cyclophosphamide (2 g/m2) with GCSF.

Methods: We performed a retrospective analysis of 79 patients mobilized with GCSF only from mid-2014 to Aug 2016 with 32 patients mobilized using high dose cyclophosphamide and 23 patients with low dose cyclophosphamide during a similar period.

Results: Patients undergoing steady state collection required a median of 2 days for adequate collection, in comparison to 1 day for both high and low dose cyclophosphamide. Addition of plerixafor was required in 27.8% of patients on steady state collection, in contrast to 31.1% and 15% of patients on high and low dose cyclophosphamide respectively. The mean yield of CD34+ x 10^6/kg cells collected was 5.39, 9.14 and 8.5 for steady state, high and low dose respectively. There was no significant difference in time to engraftment despite a lower dose of CD34+ cells reinfused for the steady state cohort. Admission for febrile episodes was observed in 50% of patients mobilized with high dose cyclophosphamide, as compared to 13% of patients on the lower dose regime and none in the steady state cohort. Patients mobilized with cyclophosphamide had a longer interval between stem cell collection and transplant (median of 20, 42 and 34 days respectively for steady state, high dose and low dose). However, we observed that 60.7% of patients with steady state mobilization had increases in their myeloma markers during this period, in contrast to biochemical improvement in 50% of patients mobilized with high dose cyclophosphamide and 26% with low dose cyclophosphamide.

Summary/Conclusions: All 3 strategies for stem cell mobilization have their 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 stem cell collection 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.
Results: Overall we analyzed 36 pts: 21 males and 14 females (median age 66, range 65-70); 23 had IgG MM, 4 had IgA MM and 9 had light chain MM. Induction therapy was bortezomib-based (bortezomib in combination with dexamethasone, VD, in 7, or VD plus thalidomide in 26 pts) for a median of 4 cycles (range 3-6), 2 patients received thalidomide plus dexamethasone (6-12 cycles). 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, 3/34 pts were in complete response/stringent complete response (CR/sCR), 19/34 in very good partial response (VGPR), 5/34 in partial response (PR) and 2/34 in stable disease (SD). The conditioning regimen consisted of melphalan 140 mg/sqm in 11 pts or 200 mg/sqm in 24 pts. A median number of 4.11 x10^6 CD34+ cells/Kg was reinfused (range 2.09-10.44). The most frequent complication was fever (9 pts) with gram negative bacteraemia documented in 3/9 and gram positive bacteraemia 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, 10/28 pts were in CR, 2/28 in PR, 10/28 in SD and 6/28 in PD. 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.

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 to ≥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 (21^version), searching for significant associations (p<0.05) with overall survival (OS) and progression free survival (PFS).

Results: 15 patients were included, with a median age of 58 years. Most patients were male (60%) and had PS ECOG 0-1 (93.3%) at presentation and primary PCL (80%). Median hemoglobin (Hb) and platelets values were 8.5 g/dl and 74x10^9/L, respectively. Median plasma cell percentage was 37.3% (peripheral blood) and 60% (bone marrow). IgG heavy chain was present in 33.3% and lambda light chains in 53.3% of cases. Most patients had total serum calcium ≥4.5mmol/L (60%), total proteins ≥65g/L (66.7%), monoclonal component ≥30g/L (53.3%), albumin ≥35g/L (60%), creatinine clearance ≥50mL/min (66.7%), elevated β-2 microglobulin (93.3%), ISS III (80%), R-ISS III (73.3%) and at least 1 cytogenetic change associated with poor prognosis in multiple myeloma (86.7%). Ten (66.7%) patients received bortezomib-based chemotherapy and nine patients (60%) were submitted, at diagnosis, to autologous stem cell transplantation (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 of 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
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Background: MM-003 study has presented a median PFS of 4.0 months and median OS was 13.1 months overall for Pomalidomide and low doses of dexamethasone in RRMM patients. Those results were better than a third drug was added (Poma-Dexa , Poma-Cyclophosphamide-dexa, and Poma-Bortezomib-dexa, ORR 38.9, 64.7 and 85%; PFS 4.4, 9.5, 10.7 months respectively).

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 had poor response. In less in the bone marrow number and third drug (cyclophosphamide or Bortezomib) and we have obtained the best results.

Results: We have used pomalidomide and dexamethasone in 4 patients and poma-dexa-cyclophosphamide in 3 patients (extramedullary myeloma) and...
Background: Plasmablastic (PB) feature of plasma cells in multiple myeloma (MM) has long been identified as poor prognosis. Interestingly it does not take part of International Revised Scoring System (R-ISS). Similarly, the prognostic impact in the era of novel agents and novel classes in MM is unknown. Finally, the percentage of PB in the bone marrow to which a poor prognosis develop is unclear.

Aims: To assess which modality of treatment of plasmablastic MM was associated with longer progression free survival (PFS) and overall survival (OS).

Methods: We have performed a retrospective analysis of all MM in our center from May 2005 to November 2016, and sought for MM with plasmablastic features, characterized by immature cells with high proliferative index rate. The PFS and OS were calculated since the first time the PB morphology was observed in the bone marrow aspiration, at the outset in newly diagnosed patients or in relapsed patients.

Results: 65 patients with PB were included. Adverse cytogenetic per IMWG criteria was reported in 6 patients, del17p x3, t(4;14) x3, and one with both. 33.8% were ISS 3, and 23.1% R-ISS 3. Extramedullary disease (EMD) was reported in 6 patients, del17p x3, t(4;14) x3, and one with both. 33.8% of patients had documented hypercalcemia, 61.5% of patients had elevated lactate dehydrogenase. We then sought to demonstrate that use of a direct anti proliferative agent such as anthracycline would participate to rapid disease reduction and OS were 6.9 and 14.9 months as a whole, respectively. The median PFS 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 entry, presence of EMD, of del(17p), of hypercalcemia, and elevated lactate dehydrogenase. The percentage of PB in the bone marrow to which a poor prognosis develop is unclear.

Summary/Conclusions: Plasmablastic, dexamethasone and a third drug (cyclophosphamide or Bortezomib) obtain best results (PFS and OS) in high risk RRMM patients. We have not reported more toxicity adding a third drug. In our experience, the response of the extramedullary myeloma with pomalidomide’s triplets is a great option.

PB1987

PROGNOSTIC SIGNIFICANCE OF PLASMA BLASTIC POPULATION IN MM

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Background: Plasma blast (PB) in bone marrow aspirate (BMA) is a known adverse feature in multiple myeloma (MM). Aims: To explore the clinical impact of PB in MM. Methods: We performed a retrospective analysis of BMA samples from 535 newly diagnosed MM patients treated according to the different treatment eras in our center. PB were scored as follows: 1 = presence of PB, 2 = greater than 5% of PB and 3 = multiple extramedullary infiltrates. The association with other adverse features was studied in a univariate analysis. Results: The relationship of PB with del(17p), ISS stage 3, hypercalcemia, presence of EMD, and elevated lactate dehydrogenase was significant in univariate analysis. In multivariate analysis, poor OS was associated to acute renal failure at disease onset, presence of EMD, of del(17p), of hypercalcemia, and elevated lactate dehydrogenase. We then sought to demonstrate that use of a direct anti proliferative-agent such as anthracycline would participate to rapid disease reduction and OS were 6.9 and 14.9 months as a whole, respectively. The median PFS entry, presence of EMD, of del(17p), of hypercalcemia, and elevated lactate dehydrogenase. The percentage of PB in the bone marrow to which a poor prognosis develop is unclear.

Summary/Conclusions: Plasmablastic, dexamethasone and a third drug (cyclophosphamide or Bortezomib) obtain best results (PFS and OS) in high risk RRMM patients. We have not reported more toxicity adding a third drug. In our experience, the response of the extramedullary myeloma with pomalidomide’s triplets is a great option.

PB1988

INTERNATIONAL OPPORTUNITIES TO COMPARE ‘REAL WORLD’ DATA FROM MYELOMA REGISTRIES: BASELINE CHARACTERISTICS, FIRST-LINE THERAPIES AND EARLY OUTCOMES FROM AUSTRIA AND AUSTRALIA/NEW ZEALAND

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Background: Most outcome data for multiple myeloma (MM) come from clinical trials which can not necessarily be extrapolated to ‘real world’ patients. More information is needed on patients treated in the ‘real world’ and in a wider range of settings.

Aims: To compare and contrast baseline characteristics, investigations, and initial therapies in different geographical regions, Australia/New Zealand (ANZ) and Austria, through first analysis of data from two established MM registries on behalf of the steering committees of the Australian and New Zealand Myeloma and Related Diseases Registry and the Austrian Myeloma Registry.

Methods: Analysis of data from newly diagnosed MM patients enrolled on the Austrian Myeloma Registry (AMR) and the ANZ Myeloma and Related Diseases Registry (MRDR) from 2012-2015.

Results: Available data from 250 and 691 patients from the AMR and ANZ MRDR, respectively, were included. DEMOGRAPHICS: The AMR cohort was younger (median age: 63.5 years/64 years vs 65 yrs/66 yrs on the AMR and MRDR, respectively). The proportion of male to female patients was similar between the AMR and MRDR (ranging from 56%/44% and 61%/39%, respectively). PRESENTATION: IgG myeloma was the most common sub-type of disease in both registries (ranging from 64%/55% and 55%/58%, respectively) with more light chain disease only on the AMR (26%/33% vs 20%/19%). Presence of documented monoclonal plasmablastic cell dyscrasias was similar (21%/19% on the AMR and MRDR, respectively). INVESTIGATIONS: A higher proportion of patients underwent MRI (31%/51%/58% vs 25%/27%) and skeletal survey (SS) (78%/60% vs 60% on diagnosis on the AMR or the MRDR, respectively). Baseline laboratory investigations were similar, however, patients on the MRDR demonstrated higher median LDH (176/178 vs 187/186 units/L) and serum calcium (2.34/2.28 vs 2.41/2.45 mmol/L) but decreased serum albumin (39/39g/L vs 35/35gL) when compared to the AMR. STAGE: ISS staging was similar on both registries with ISS stage 2 being most common in both cohorts (42%/37% vs 40%/40%, on the AMR and MRDR, respectively) while ECOG performance status at diagnosis was lower in the MRDR cohort (ECOG1, 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%/85%). Dexamethasone (D) was the most common on the AMR, while V/dexamethasone/D (VTD) (25%) was used most on the MRDR. Combination of V with thalidomide, lenalidomide (VTD/VTCD) (43%/49%) versus only V (21%/24%) was greater on MRDR. In both registries, IMiD (lenalidomide and thalidomide) was administered more frequently (57%/58%) than alkylator (40%/42%) on the MRDR. In the AMR, IMiD and alkylator were first line in 34%/36% of patients while IMiD and alkylator were less common on the MRDR (15%/20%). Differences in regimen were noted between various ISS stages but the effect was not statistically significant. CONCLUSION: The predominant therapy in newly diagnosed MM patients is V/alkylator/IMiD (VTD/VTCD). Further studies are required to determine patient specific factors that influence treatment choice and the clinical benefit of current therapies.

Figure 1.
weeks and every 3 months later (minimum follow-up: 6 months). EBR was defined as 25% on M-protein increase (any amount for patients on CR/SR) and/or ≥20mg/dl FLC increase, and/or 25% involved HLC 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. MRatio: 29/26, mean age 59.5 ± (33-71). Immunoglobulin subtype: IgG: 41.8% (23), IgA: 23.6% (13), IgD: 6% (3), IgM: 16.4% (9), IgA-Lambda: 7.3% (4), Bence-Jones-Lambda: 3.6% (2), Bence-Jones-Kappa: 7.3% (4). Durie-Salmon Stage: IA: 13.5% (7), II-A: 32.7% (17), III-A: 44.2% (23), III-B: 9.6% (5), missing-data 3 case. All patients received Bortezomib based therapy and MEL200 as ASCT conditioning. Status pre-ASCT: minimal response: 22%, Partial Response (PR): 50.0%, very-good-PR (VGPR): 28.0%, complete response (CR): 6% and string response (SR): 4.0%. After ASCT, evaluation reveals that 13.0% achieved SR, 30.4% VGPR and 39.1% PR. During follow-up, 34/50 (68%) patients who achieved at least PR after ASCT, had a clinical relapse/progress, median PFS 41 months (31.5-50.5). EBR were detected in 28 patients, of them 22/34 (64.7%) clinically relapsed patients at median time 8.0 (2-22) months before symptomatic relapse. The EBR were detected by FLCr (36.7%), HLCr (22.7%), FLC+Spe (4.5%), FLC+IFX (9.1%), FLC+HLC+Spe (13.6%), FLC+HLC+Spe+UPE (13.6%).

**Summary/Conclusions:** Both FLC and HLC are useful tools to detect EBR in more than 50% of patients in our cohort ahead other techniques.

**PB1990**

**EARLY MORTALITY (<6 M) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS: COMPREHENSIVE INTERVENTION**

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**Background:** Early mortality in the first 6 to 12 months from diagnosis is well recognized in newly diagnosed multiple myeloma (NDMM) patients, with rates in the range of 2% to 6%.

**Aims:** In a retrospective analysis of the causes of death performed by the end of 2012 we identify 2 different causes in the 2 consecutive periods analyzed. In the first period (1998-2006) the main cause was MM progression and in the second (2006-12) was secondary to serious infectious complications. Additional analysis were done after it an can identify a patient and infectious profiles. Main risk factors from the patient were: age (over 75), suboptimal treatment and renal failure (calculated Ccr<50 ml/min). The infectious mainly occurred the first 3 months from diagnosis and principally polymicrobial and multiresistant infections.

**Methods:** After this analysis several measures were taken to reduce this high early mortality: 1) To promote the ambulatory regime both in diagnosis and for the rapid assessment of complications to avoid or shorten income and to reduce these nosocomial-behaviour infection complications. 2) Early initiation of "optimal" anti-myeloma treatment. 3) Get infectious prophylaxis in patients over 75 years and or renal failure with Septrim ©.

**Results:** For urine, an increase >500mg/24 hrs of involved free-chain protein.

**Summary/Conclusions:** Infectious complications and progression of MM have been the main cause of early mortality in patients with NDMM. Identifying potentially "modifiable" variables and acting on them improves the short-term prognosis of patients with NDMM like: Supportive treatment to prevent infectious complications (avoid unnecessary hospitalization, antibiolic prophylaxis) and rapid access to optimal antiMM treatments. These improvements of short-term

**PB1991**

**FIRST LINE USE OF NOVEL AGENTS BEFORE AUTOLOGOUS SCT HAS A POSITIVE IMPACT ON TIME TO SECOND PROGRESSION AND SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA UNDER 70 YEARS**

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**Background:** Most clinical trials for multiple myeloma (MM) patients using novel agent-based regimens before autologous stem cell transplantation have shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.

**Aims:** Our aim is to analyze the potential impact of initial induction in the feasibility of these same agents second line. Indeed, these patients also had the case. The use of thalidomide and/or bortezomib induction did not reduce the efficacy of these same agents second line. Therefore, these patients also had the best second responses that also contributed to a longer PFS2 periods. After a median follow-up of 6.97 years, clear differences in OS were observed (7.97 years for NA compared to 3.35 years in CC, P<0.001). Despite the fact that better risk patients in the NA group were more likely to remain in first or second response, relapsed and refractory patients in this group still presented longer survivals beyond second relapse than patients in the CC group (Figure 1).

**PB1992**

**SAFETY AND EFFICACY OF NOVEL AGENTS IN VERY ELDERLY MULTIPLE MYELOMA PATIENTS (AGED 80 YEARS OR MORE): A REPORT BY THE RETE EMATOLOGICO PUGLIESE (REP)**

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**Background:** First line use of novel agents before autologous SCT has shown improvement in response rates and progression-free survival, however they have failed to identify a significant overall survival (OS) benefit.

**Methods:** We report the results of 343 MM patients aged 80 or more years treated between 1998 and 2015 (127 in the first period, 115 in the second period, 103 in the third period).

**Results:** The overall response rate after completing first line treatment for all the 154 eligible patients was 85%, 79% in CC compared to 94% in NA (P=0.012). Very good partial response or better for NA was significantly higher than for CC (39% vs 29%, P=0.012). Patients in NA demonstrated not only a superior median progression-free survival (2.8 years vs 1.6 years, P=0.03) but also a superior median progression-free survival from diagnosis to second progression – PFS2 (5.2 years vs 2.7 years, P=0.003). In both cohorts PFS1 and PFS2 represented more than 50% and 80% of life expectancy respectively. It could be hypothesized that CC patients would obtain more benefit than NA patients of second-line therapy, as they would be naïve to the novel agents used at relapse, but this is not the case. The use of thalidomide and/or bortezomib induction did not reduce the efficacy of these same agents second line. Indeed, these patients also had the best second responses that also contributed to a longer PFS2 periods. After a median follow-up of 6.97 years, clear differences in OS were observed (7.97 years for NA compared to 3.35 years in CC, P<0.001). Despite the fact that better risk patients in the NA group were more likely to remain in first or second response, relapsed and refractory patients in this group still presented longer survivals beyond second relapse than patients in the CC group (Figure 1).

**Summary/Conclusions:** New agent based first line induction treatments in newly diagnosed MM patients provide benefits beyond first progression free survival that contribute to a significant improvement in OS.
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 of all MM patients. This subset of patients suffers 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 (M/F: 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-λ (n=16), IgA-k (n=14), IgA-λ (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 bone pain and renal failure were found in 35 (49.3%) 2 (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 (n=11; 16.4%) and Pomalidomide-based (n=8; 11.3%) regimens. 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 VGP 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 28 (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. Neupathy was the most common toxicity reported (n=5, 7%). Of 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.

PB1993

BORTEZOMIB-MELPHALAN-PREDNISONE VERSUS MP AS INITIAL TREATMENT FOR VERY ELDERLY PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA


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Background: Although bortezomib-melphalan-prednisone (VMP) therapy is a well-established combination for patients with newly diagnosed multiple myeloma (MM) who are ineligible for high-dose therapy, it is not clear whether very elderly patients should be treated with VMP in clinical practice, considering the toxicities.

Aims: The purpose of this case-control study was to compare the efficacy of VMP versus melphalan-prednisone or cyclophosphamide-prednisone (MP/CP) as initial therapy for elderly patients.

Methods: We retrospectively studied 233 patients aged 75 years or older with newly diagnosed multiple myeloma between March 2007 and February 2015. One-hundred thirty one patients received VMP and 102 patients received MP/CP regimen were enrolled from 15 institutions throughout Korea.

Results: Patient characteristics were comparable in these two groups. Overall response rate was 70.2% in VMP patients and 48.0% in MP/CP patients (P=0.001). Complete response rate was 22.9% in VMP patients and 7.8% in MP/CP patients (P=0.002). After a median follow-up for survivors of 28.5 months, progression-free survival (PFS) and overall survival (OS) were significantly different between the two groups (PFS, median 15.5 vs.11.8 months in VMP and MP/CP group, respectively, P=0.018; OS, median 34.9 vs. 22.8 months in VMP and MP/CP group, respectively, P=0.006). Nonetheless, for 61 patients who were aged ≥80 years, PFS and OS was not significantly different between the two groups (PFS, median 19.6 vs 13.2 months in VMP and MP/CP group, respectively, P=0.376; OS, median 27.8 vs.17.8 months in VMP and MP/CP group, respectively, P=0.443).

Summary/Conclusions: Although VMP therapy was associated with a significant improvement in overall survival among patients ≥75 years, there is no differences for patients aged 80 or older. Frailty and comprehensive geriatric assessment should be incorporated to guide treatment decisions for this population.

PB1994

EFFICACY OF AUTOLOGOUS STEM CELL TRANSPLANTATION FOR THE TREATMENT OF MULTIPLE MYELOMA IN HIV-POSITIVE PATIENTS – A CASE-SERIES

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Background: While hematopoietic malignancies are found at increased rates in individuals with acquired immunodeficiency syndrome (AIDS), the presence of multiple myeloma (MM) and human immunodeficiency virus (HIV) is less common, leading to a paucity of expertise in the treatment of individuals with these co-morbid conditions. Prior to the advent of highly active anti-retroviral therapy (HAART), autologous stem cell transplant (ASCT) was relatively contraindicated for MM patients with HIV due to issues associated with stem cell harvest and the risk of opportunistic infections. With the widespread use of HAART for control of HIV, high dose chemotherapy and ASCT is now the preferred treatment for relapsed lymphoma, the leading hematopoietic malignancy associated with HIV/AIDS. It stands to reason that MM patients with HIV on HAART may benefit equally from aggressive combination treatment of chemotherapy and ASCT.

Aims: This study seeks to evaluate the clinical course and treatment outcomes of patients with HIV and MM treated with high dose therapy and ASCT.

Methods: A single center retrospective case-series study was performed. Data from patients who were HIV-positive and on HAART undergoing ASCT for treatment of MM between January 2000 and June 2016 were collected and analyzed.

Results: The following Table 1 lists patient characteristics. All were male with average age 53.2 years. All were diagnosed with HIV prior to diagnosis of MM and were appropriately treated with HAART prior to ASCT. All patients had a CD4 count <200/uL prior to ASCT and most remained undetectable after ASCT. Four of five patients had CD4 >200/uL and one patient had CD4 <50/uL prior to ASCT; however all patients recovered CD4 counts after ASCT (and most with improved CD4 count). Adequate CD34(+) stem cells were collected. Patients received high dose melphalan (200 mg/m2) followed by ASCT. HAART was continued during ASCT. Patients experienced usual ASCT toxicities including diarrhea, mucositis, and neutropenic fever. One patient developed sepsis and small bowel obstruction, which resolved with antibiotics and conservative management. All patients had normal neutrophil and platelet engraftments. Post-ASCT responses were complete remission (2 patients), very good partial remission (1), partial remission (1) and minimal response (1). All patients are currently alive without relapse or progression 1-4 years from ASCT and receiving post ASCT maintenance with lenalidomide.

Table 1.
Summary/Conclusions: Multiple myeloma patients with concurrent HIV infection that is controlled on HAART tolerate ASCCT for treatment of myeloma as well as myeloma patients without HIV infection and have generally good outcomes.

PB1995
FEASIBILITY OF USING GLOBAL FDG UPTAKE IN BONE MARROW TO ASSESS TREATMENT RESPONSE IN MULTIPLE MYELOMA
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Background: Multiple myeloma (MM) is characterized by plasma cell proliferation and expansion primarily in the bone marrow. Modern assessment of MM using FDG-PET has so far been limited to the analysis of focal lesions, requiring subjective interpretation to determine overall disease activity.

Aims: A novel method using CT segmentation to determine global bone marrow activity portrayed by FDG uptake was used to achieve a comprehensive understanding of disease burden in patients with MM before and after therapy.

Methods: Prospective FDG-PET/CT data of 23 MM patients between ages of 50 and 76 (mean=64.3, males=21, females=2) were collected from Odense University Hospital (NCT02187731) and included scans before initiation of treatment and at end of treatment (EOT) two months after high dose chemotherapy with stem cell support. All scans were conducted 60 min after intravenous injection of 418 MBq of FDG. Images were analyzed using an iterative thresholding algorithm that delineates a continuous region based on Hounsfield units from the CT data (OsiRx software; Pixmeo SARL; Bernex, Switzerland), allowing for segmentation of the total skeleton on a fused PET/CT image. This enabled the quantification of FDG uptake representing the entire skeleton, providing a global SUVmean that considers all bone marrow involvement. Global SUVmean scores were compared before and after EOT using a two-tailed paired t test.

Results: A decrease in marrow FDG uptake was observed at EOT compared to baseline in most patients. The calculated global SUVmean uptake decreased after initiation of treatment in 17 (73.9%) of the cases and increased in 6 (26.1%) of the cases, as determined by the observed statistical difference of the dependent means before and after treatment (P=0.0053).

Summary/Conclusions: We assessed the effects of treatment in MM patients using a novel technique for global quantification of FDG uptake in the bone marrow and skeleton and found lower global uptake at EOT. However, a limitation of this method is that the presence of fractures, bone biopsy or any other cause and time to next treatment (TNT), defined as time from transplant to next new therapy or death of any cause.

Results: From January 2012 to December 2016 hundred and one patient with MM (49 male, 52 female), median age 55 (range 22-71), were transplanted. Bortezomib based induction therapy was used in 55 (54.5%) and VAD induction was used in 46 (45.5%) patients. Median OS of all treated patients was 73 months; median OS of VAD group was 73 months while in bortezomib group median OS was not reached, but this difference was not statistically significant (P=0.19). TNT was significantly longer in bortezomib group than in VAD one (27.8 vs 17.5 months respectively; p=0.02). Interestingly prognostic indices could not discriminate patient groups according to OS (p=0.1), but could discriminate them due to TNT (p=0.008), possibly due to cross-over to bortezomib treatment after treatment failure. TNT had a significant correlation with levels of LDH (p=0.04) and no significant correlation with number of plasma cells in bone marrow. OS was significantly longer in those with longer duration of time to next treatment (p=0.0004). There was no difference in OS or TNT in patients treated with tandem transplant vs single transplant (p=0.68 and p=0.57 respectively), possibly due to heterogeneity of tandem group.

Summary/Conclusions: Even though novel drug therapy seems to converge risk groups to lower ones, prognostic indices remain relevant. Due to heterogeneity of patients and myriad of known prognostic factors further studies are needed so they may be translated into risk adapted therapy approach.

PB1997
WHICH ORGAN SHOULD WE BIOPSY TO DIAGNOSE AL AMYLOIDOSIS?
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Background: Light chain (AL) amyloidosis is a deposition disease with can affect many organs and with a variable but usually bad, prognosis. Therapy requires a quick and correct diagnosis. Accurate identification of amyloid deposit and of the amyloid subtype in tissue biopsies is thus, mandatory. Random biopsies of easily accessible tissues such as subcutaneous fat, gingivae or rectum are usually recommended but sensitivity of this approach is low.

Aims: To present our experience with tissue biopsies performed in 62 consecutive patients diagnosed of AL amyloidosis in our center.

Methods: We reviewed all tissue biopsies performed during the study period (2004-2017) in 62 consecutive patients diagnosed of AL amyloidosis at the same center. A bone marrow (BM) biopsy was performed per protocol in all cases. Decisions on biopsies were taken considering organ involvement and accessibility. Skin, lymph nodes, lung or tongue biopsies were performed when lesions were seen on clinical or X-ray examinations, cardiac biopsies in the presence of increased NT-proBNP (N-terminal natriuretic peptide) levels and typical echocardiographic findings, kidney biopsies in patients with nephrotic syndromes. Biopsies were stained with Congo Red and read under polarized light with a Texas filter. Subtyping of the amyloid was done using anti-kappa, anti-lambda, anti-TTR and anti-A antisera. If any biopsy was positive for AL amyloid, no further biopsies were performed unless necessary for therapeutic decisions.

Results: A total of 152 biopsies were performed during the study period: see Table 1.

Table 1.

Summary/Conclusions: Prognosis in AL amyloidosis is slowly improving with the use of new anti-myeloma drugs and may improve further with monoclonal antibodies. Therapy requires an early and accurate diagnosis. We do not perform random biopsies of tissues such as fat or gingivae due to low sensitivity. In our hands, biopsies of tissues such as rectum are usually recommended but sensitivity of this approach is low.

Table: 152
PB1998

A COMPARISON OF CYCLOPHOSPHAMIDE-GLUCOCORTICOIDS AND LENALIDOMIDE-DEXAMETHASONE AS TREATMENT FOR MULTIPLE MYELOMA IN FIRST RELAPSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background: The optimal management of relapsed Multiple Myeloma (MM) with respect to therapeutic combinations and sequence remains controversial and is actively evolving. Many commonly used regimens have not been directly compared. These agents vary widely in cost, and knowledge of their relative efficacy is of particular importance in regions where cancer medicines are publicly funded.

Aims: We sought to compare the efficacy and safety of two commonly used regimens for relapsed MM using historical cohorts from a single transplant center.

Methods: A retrospective observational study was performed between January 1991 and November 2016 to compare the efficacy of cyclophosphamide and dexamethasone/prednisone (Cyclo), or lenalidomide and dexamethasone (Len-Dex) for relapsed MM post autologous stem cell transplant (auto SCT). The primary outcome was Time to Next Treatment 2 (TTNT2), defined as time from first relapse requiring therapy after auto SCT to second relapse requiring therapy. The secondary outcome was overall survival, defined as time of diagnosis to death from any cause. Outcomes were assessed by Kaplan Meier methods and overall differences determined by log rank test. Hazard ratios were calculated for individual treatment groups and compared by univariate and multivariate logistic regression.

Results: A total of 243 patients underwent treatment for MM at first relapse post autologous transplant. Of these, 139 were included in this analysis: 88 Cyclo and 51 Len-Dex. Patient demographics and disease characteristics were similar between each group for age, sex, subtype of MM and ISS Stage. 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% observed only in 8 patients. We estimated the AUC0-24 of Len by using formula 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 2513.8ng•hr/ml (sensitivity 81.8%, specificity 80%) and non-hematologic AEs 3023.6ng•hr/ml (sensitivity 78.9%, specificity 62.5%).

PB1999

CLINICAL IMPACT OF THE PLASMA LENALIDOMIDE CONCENTRATION AND THE ANALYSIS OF ANTI-TUMOR IMMUNE RESPONSE IN NEWLY DIAGNOSED MULTIPLE MYELOMA TREATED WITH LENALIDOMIDE AND DEXAMETHASONE THERAPY

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Background: Lenalidomide (Len) and dexamethasone (DEX) combination therapy is now the standard treatment of multiple myeloma (MM). Len has both a direct effect on MM cells and an immunomodulatory effect and recently many drugs are combined with Ld therapy to expect the synergistic anti-tumor immune response. However, adverse events (AEs) make continuation of Ld therapy difficult for some patients especially for elderly patients.

Aims: To investigate the safe and effective plasma concentration of Len and the anti-tumor immune response change in MM patients treated by Ld therapy.

Methods: Forty patients (18 men and 22 women) were enrolled in this study. Median age was 75.5 years old (range 61-86). Len was administered on days 1–21 of a 28-day cycle; and DEX, on days 1, 8, 15, and 22. The plasma concentrations of Len just before oral administration and 1, 2, and 4 hr thereafter were analyzed by using 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 antigens and intracellular antigens or intracellular cytokine production of IFN-γ, TNF-α, IL-2 and CD107a molecule was detected after stimulation with PMA/ionomycin for 5 hours in the presence of protein transport inhibitor Golgi stop (BD Bioscience). Analysis was performed using LSR Fortessa (BD Bioscience) and Flowjo version 10.2 software (TreeStar). This study protocol was approved by the Ethics Committee of Akita University Hospital, and all recipients gave written informed consent.

Results: 21 patients showed renal impairment (RI) necessary to adjust initial Len dosage. Adverse cytogenetics of del17p and t(4;14), detected by using fluorescence in situ hybridization, were found in 2 and 4 patients, respectively. The median initial dosage of Len was 15 mg and DEX 20 mg. The overall response rates were 68.6% and the 2-year progression-free survival was 70.8% at a median follow-up of 26.5 month. Grade 3 to 4 nonhematologic AEs were observed only in 8 patients. We estimated the AUC0-24 of Len by using formula as we previously reported (Ther Drug Monit 2014) and the cut-off value of the hematologic AEs was 2513.8ng•hr/ml (sensitivity 81.8%, specificity 80%) and non-hematologic AEs 3023.6ng•hr/ml (sensitivity 78.9%, specificity 62.5%). After Ld therapy, naive 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, Figure1). Expression of CD56 on the surface of extramedullary MM cells was found in 76.5% patients. OS in the group of patients with CD56+ in extramedullary MM cells and in bone marrow cells (n=9) was 67% which was significantly higher (p=0.04) than that in the group of patients (n=4) with CD56+ in extramedullary MM cells and CD56- in bone marrow cells – 50%. Simultaneous lack of CD56 in extramedullary MM cells and in bone marrow cells was found 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**

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**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 1,2,4,8,9,11,12, Pegfilgrastim day +4) every 28 days, until progression. ISS was equally distributed, and cytogenetic was evaluable in 12 patients, including 1 patient with del13q and one t(11;14). All the patients had previously been treated with scheme 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 resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

**Results:** According to IMWG, after a median follow-up of 14 months (r.2-36), ORR was 64% (36/56 : 4 CR, 7 VGPR, 16 PR, 9 MR) with 8 PD and 12 patients in SD, which can be considered as an impressive result in this subset of rMM patients. In particular, for 11 patients, BVD was, after having achieved at least a PR, a bridge to second autSCT, and for two patients a bridge to alloSCT. Median time to response was 1.2 months (r.1-3), median OS from diagnosis was 62.7 months (range 6-151), median OS from start of Bendamustine was 9.8 months (range 2-36).

**Table 1.**

<table>
<thead>
<tr>
<th>Total patients</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>31</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
</tr>
<tr>
<td>Median age, years</td>
<td>57.3 (36-82)</td>
</tr>
<tr>
<td>at diagnosis, (range)</td>
<td>61.8 (48-73)</td>
</tr>
<tr>
<td>Previous regimen</td>
<td>6 (2-11)</td>
</tr>
<tr>
<td>FISH analysis</td>
<td>12/56</td>
</tr>
<tr>
<td>negatve</td>
<td>10</td>
</tr>
<tr>
<td>del13q</td>
<td>1</td>
</tr>
<tr>
<td>(t11;14)</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Previous therapies: no. of patients(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib</td>
</tr>
<tr>
<td>IMIDs</td>
</tr>
<tr>
<td>Autologenic SCT</td>
</tr>
</tbody>
</table>

**Summary/Conclusions:** BVD has shown significant efficacy in a particularly severe setting of patients, relapsed and refractory to all available therapeutic resources, and, in particular cases, it could be considered as a bridge to a second autologous or allogenic SCT.

**PB2002**

**VE-CADHERIN IN MULTIPLE MYELOMA: AN INDEPENDENT PROGNOSTIC FACTOR FOR PROGRESSION-FREE SURVIVAL**

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**Background:** Endothelial damage and perivascular infiltrates are vital in the development of multiple myeloma. Recent studies have found that endothelial dysfunction might be result in multiple myeloma progression and adverse effects of drug implementation. On the other hand, there is a direct correlation between microvessel density in multiple myeloma and parameters of disease progression. Endothelial cells participate in inflammatory events leading to apoptosis by regulating entry via the endocytosis vascular endothelial-cadherin (VE-Cadherin) on their surface. VE-cadherin is cell adhesion molecules localized at the endothelial junction, which plays critical roles in angiogenesis, neovascularization, neoplasm development, stem cells mobilization, and endothelial integrity. Indeed, VE-cadherin chiefly organizes the opening and closing of the endothelial barrier. It was evaluated in 12 cadets, 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.

**Methods:** Aimed to evaluate the metabiotic prognostic value of circulating VE-cadherin for progression-free survival in patients with multiple myeloma in complete or partial remission.

**Summary/Conclusions:** To evaluate the impact of CD56 expression on the rate of overall survival (OS) in MM patients with extramedullary disease (EMD).
enrolled in the study. Diagnosis and staging of multiple myeloma were defined by current clinical practice guidelines. To be achieving remission chemotherapy with bortezomib, thalidomide, dexamethasone, cyclophosphamide, melphalan, and anthracyclines was used accordingly contemporary clinical guidelines. All subjects were at complete or partial remission at baseline. Observation period was up to 12 months. ELISA method for measurements of circulating level of VE-cadherin was used.

Results: Medians of circulating levels of VE-cadherin in subjects without progression of multiple myeloma (n=89) and subjects with progression (n=23) during 12 months were 0.92 ng/ml (95% confidence interval [CI]=0.66-1.19 ng/ml) and 1.77 ng/ml (95% CI=1.47-2.07 ng/ml) (p=0.0002). The best VE-cadherin cutoff for predicting progression-free survival was 1.31 ng/ml, with AUC value 0.839 (p=0.0001), the sensitivity and specificity were 77.8% and 61.5% respectively. The presence of high levels of serum VE-cadherin was significantly correlated to a shorter progression-free survival (PFS). In a multivariate analysis along with clinical and biologic prognostic parameters, high serum VE-cadherin level (≥1.31 ng/ml) was an independent adverse prognostic variable for PFS (median PFS 9.93 (IC=8.16-11.71) months vs 7.35 (IC=5.75-9.85) months (p=0.02).

Summary/Conclusions: The serum VE-cadherin level is a valuable biomarker for predicting treatment response and an independent prognostic factor for progression-free survival for patients with multiple myeloma.

PB2003

THE UTILITY OF FACS PURIFICATION OF PLASMA CELLS FOR FISH ANALYSIS IN MONOCLONAL GAMMAPATHIES

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Background: Despite the prognostic value of chromosomal aberrations, conventional metaphase karyotyping in monoclonal gammapathies (MG) is often informative, mainly 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 guarantee the confident application of all 5 probes in our lab.

Results: After the exclusion of samples diluted with peripheral blood, we identified 102 patients with FACS separated purified PC. An average of 165 393±270 516 PC were separated per patient, and 98 of the cohort (96.1%) had a sufficient number of cells for the hybridization of at least one FISH probe; all 5 probes were applied in 30% of patients, 4 in 50%, 3 in 12% and 2 in 8%; the motives underlying the selection of fewer than all 5 probes in samples with a sufficient number (>20 000) of cells included the individual decision of the assisting physician and, for t(14;16), the date of the study. Considering only those studies performed after the introduction of t(14;16), all 5 probes were used in 67.6% of cases; 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(1p32), 5.6% (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 2.9% (p=0.006) in the other group. Both del(1p32) (5.6% vs 5.6%, p=NS) and amp(1q21) (46.3% vs 33.3%, p=NS) were identically distributed across diagnoses. 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.

Summary/Conclusions: We have found that the application of FISH probes in FACS-separated PC is highly efficient with a robust yield, providing a large enough sample for the application of at least two probes in over 95% of patients, irrespective of bone marrow plasmacytosis; in fact, we obtained an average of 165 000 pure PC per patient, which is more than 8-fold higher than the number we consider invariably sufficient to apply 5 probes, which we achieved in at least 80% of patients.

PB2004

CLINICAL SPECTRUM AND EVOLUTION OF MONOCLONAL GAMMAPATHY ASSOCIATED NEUROPATHY VERSUS CHRONIC INFLAMMATORY DEMYELINATING POLYNEUROPATHY PATIENTS

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Background: Paraproteinemic neuropathy (PPN) refers to a disorder of the peripheral nervous system associated with a monoclonal gammopathy. It is known that about 10% of idiopathic peripheral neuropathies are of this type. Unfortunately, PPN is often underdiagnosed or confused with chronic inflammatory demyelinating polyneuropathy (CIDP), subsequently leading to inappropriate management. Since progression of neuropathy is associated with possible malignant conversion of underlying monoclonal gammapathy, it is important to recognize underlying hematological conditions.

Aims: We aimed to determine whether the clinical characteristics and course differed in patients with PPN compared to those with CIDP in order to identify factors useful for differential diagnosis.

Methods: This study was carried out at Seoul National University Hospital, which is a tertiary academic center. During the period between January 2005 and December 2016, patients with 1) monoclonal gammapathy of undetermined significance (MGUS), and 2) CIDP were identified. Those with previous history of cancer or autoimmune disease requiring treatment with immunomodulatory agents were excluded from analyses. In the end, a total of 18 MGUS patients and 34 CIDP patients, with complete set of data including clinical physical examinations, electrodiagnostic studies, and laboratory test results, were enrolled.

Results: In both groups, males were predominant. IgG MG was most common (55.6%) in our cohort. PPN appeared to be mainly sensory regardless of heavy chain or light chain. Compared to PPN patients, CIDP patients were associated with motor symptoms manifesting as motor weakness (50.0% vs 91.2%, P=0.001) and ataxia (44.4% vs 61.8%, P=0.043) (Table 1). There were equal number of axonal type neuropathy and demyelinating type neuropathy in patients with PPN, and there were no differences in type of neuropathy between various immunoglobulin subclasses. However, demyelinating type PPN was associated with more severe clinical presentations, including more dysesthesia, pain and sensory symptoms. During median follow-up of 49 months, 2 PPN patients developed overt hematologic malignancies: 1 case of Waldenstrom macroglobulinemia and 1 case of AL amyloidosis. Among them studied malignant transformation within 8 months of neuropathy development, and were associated with worsening neuropathic symptom at the diagnosis of hematologic malignancy. There were no differences between the two groups with regards to overall survival.

Table 1. Clinical characteristics of all enrolled patients.
Summary/Conclusions: Although both PPN and CIDP patients suffer from 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 treatment strategy.

Aims: Finding molecular genetic criteria of predicting the effectiveness of autologous peripheral blood hematopoietic stem cell transplantation (auto-PBHSCT) for improving algorithm of multiple myeloma (MM) patients cure at various stages of treatment.

Methods: The study involved 61 patients with MM and relapse and primary therapy resistant patients. Molecular cytogentic, immunogenetic, hematological and statistical methods were used.

Results: Since appearance of genetic abnormalities in the malignant plasma cells is one of the pathogenic mechanisms of the disease, genetic support of patients is essential. It was determined that the carriage of the allele HLA-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-PBSCST is associated with carriage of haplotype HLA-C*06 - HLA-DQA1*01: 01 (F=4.87, p=0.028; OR=7.34, p=0.05). Abnormalities of chromosomes 4, 11, 13, 14 and 17 were determined in 35 of 61 (57%) MM patients with complicated disease course and minimal therapy response. Significant alterations were revealed in the presence of two or more abnormal clones (23 patients (37.7%), Ro Spirmann=0.42, p < 0.05), deletion of chromosome 17 (17 patients (27.9%), Ro Spirmann=0.41, p < 0.05), deletion/monosomy of chromosome 13 (10 of 15 patients surveyed, Ro Spirmann=0.33, p < 0.05), the translocation t(4;14) (4 patients (6.6%), Ro Spirmann=0.50, p < 0.02).

Summary/Conclusions: The results indicate the necessity of introducing the molecular genetic support into protocol of examination MM patients on various stages of treatment with auto-PBHSCT.

PB2006

THE INFLUENCE OF MINIMAL RESIDUAL DISEASE AND TUMOR LOAD ON THE PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA PATIENTS

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Background: Use of modern drugs and their combinations in the complex antitymomega therapy (induction, high-dose therapy (HDT) with autologous stem cells 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 (V D, VD, VMP, PAD) was used in 36/52 (69%) patients, Immunomodulator-based regimens (Thal+D, RD, VRD, PomD) – in 14/52 (27%), chemotherapy – in 2/52 (4%). ASCT is carried out 31 (59.6%) patients. Primary tumor cells phenotype and MRD were detected by 5-color flow cytometry. Clonal plasmatic cells were detected by markers: CD38, CD138, CD45, CD19, CD20, CD27, CD195. 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% (9/29), 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-negative group of patients (n=36: 21 CR, 6 VGPR, 9 PR) was 21 months, in the MRD-positive group (n=16) – 6 months (p=0.006). The tumor load is also a strong prognostic factor like MRD status. Patients who attained low-level MRD had a benefit in the duration of PFS:<0.01% - 66 months, 0.01%-<0.1% - 48 months at 0.1%-< 22 months, >1% - 10 months (p=0.0009) (Figure 1).

Figure 1. The influence of tumor load on progression free survival.

Summary/Conclusions: The frequency of achievement MRD-negative doesn’t depend from program of induction therapy, HDT with ASCT and maintenance therapy. Negative prognostic role of MRD status independent from clinical response. Presence of MRD after treatment to associated with decrease of PFS and early relapse. Control of MRD allows to increase of PFS and can be done by means of modern drugs and its combinations. HDT with ASCT and maintenance therapy. Impact of MRD requires further studies, especially after HDT with ASCT.

PB2007

QUALITY OF RESPONSE AS PREDICTOR OF SURVIVAL AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN REAL LIFE MULTIPLE MYELOMA PATIENTS IN A SINGLE INSTITUTION

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is the standard treatment approach for younger patients with multiple myeloma (MM). Since the introduction of proteasome inhibitors and immunomodulatory drugs in MM treatment more patients achieve deep and durable responses and better disease control before ASCT.

Aims: To evaluate the association between the depth of response before ASCT and survival outcomes in a cohort of patients with MM.

Methods: Retrospective analysis of patients with MM treated with HDC and ASCT between 2007 and 2016 in a single institution. All patients received at least one autologous hematopoietic support regimen either 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 by 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%). 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
5 years was 83.8% and 68.9% and PFS was 74.8% and 37.3%, respectively. Before ASCT, 101 patients (51.8%) achieved very good partial response (VGPR) or better (≥VGPR) and 94 patients (48.2%) a partial response (PR). The patients in ≥VGPR presented significantly longer OS (median OS not reached vs 96.9 months, p=0.023) and PFS (58.5 vs 41.2 months, p=0.003) compared with those in PR. At 100 days after ASCT, 107 patients (54.9%) presented ≥VGPR, 79 (40.5%) PR and 7 (3.6%) progressive disease. Two patients were not assessed due to loss of follow-up. The group of ≥VGPR showed superior OS (median OS not reached vs 72.4 months, p=0.023) and PFS (58.5 vs 34.7 months, p=0.007) compared to the PR group. We did not find statistically significant differences in survival of patients who achieved ≥VGPR before or after ASCT. Univariate analysis indicates that depth of response before and after ASCT (≥VGPR vs PR) are significant predictors of OS (HR 0.49; 95% CI 0.31-0.80, p=0.004) and OS (0.49; 95% CI 0.30-0.81, p=0.005) and PFS (HR 0.50; 95% CI 0.27-0.92, p=0.026 and HR 0.49; 95% CI 0.27-0.90, p=0.021). Multivariate analysis revealed that these factors retain their prognostic value after adjustment for age, International Staging System stage and number of previous lines of treatment.

Summary/Conclusions: These findings provide evidence for quality of response as a predictor of OS and PFS after ASCT in patients with MM. Outcomes after ASCT seems to be better for MM patients who achieve deep responses (at least VGPR) before or after transplant. Our results support the use of more effective induction regimens in order to improve initial response as this may correlate with higher response rates and survival post-ASCT.

PB2008
LEPTOMENINGEAL INFILTRATION SCREENING SHOULD BE PERFORMED IN PATIENTS DIAGNOSED WITH PLASMA CELL LEUKAEMIA

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Background: Plasma cell leukaemia (PCL) is a rare and aggressive plasma cell (PC) disorder characterized by the presence of circulating plasma cells. PCL can either originate de novo (PCL) or as secondary PCL (sPCL) in patients with relapsed/refractory multiple myeloma (MM). PCL has a more aggressive clinical presentation than MM with a more frequent extramedullary involvement, such as leptomeningeal infiltration. However, because of the low incidence of this entity, most clinical data come from small retrospective studies. Clinical diagnosis criteria of PCL are today under review and the incidence of leptomeningeal infiltration is unknown.

Aims: We aimed to study the clinical features with special emphasis in the incidence leptomeningeal infiltration in patients diagnosed with PCL in our centre.

Methods: Seventeen patients were diagnosed of PCL between 2008 to 2016 in our centre. PCL was defined based on criteria from the Chronic Leukaemia: Myeloma Task Force, by the presence of ≥2x109/L peripheral blood PC or plasmacytosis accounting for more than 20% of the differential white cell count. Medical records were retrospectively reviewed. Clinical response was evaluating per IWGM criteria. Clinical and biological features, progression free survival (PFS) and overall survival (OS) were analyzed. Survival curves were estimated using the Kaplan-Meier method and comparing the Log-Rank test.

Results: Seventeen patients with PCL were included. Six (35.3%) were pPCL and eleven (64.7%) sPCL. Median age at diagnosis was 57 years (range 35-78) and 8 (47.1%) were males. Clinical and analytical features at the moment of diagnosis are recorded in Table 1.

Table 1.

Five (29.4%) patients presented with leptomeningeal infiltration; in three of them it was diagnosed at the time of the diagnosis of PCL. All the patients had neurological features. Thirteen (76.4%) patients were able to start a curative treatment: VD in 7 (53.8%) patients, VTD in 2 (15.4%), VAD in 1 (7.7%), D-PACE in 1, MTX-ARAC in 1 patient and 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, 2 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.

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.

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.

Aims: 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 treatment 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: 2 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 were responsible for initiating MM Tx. Data from 3 countries with limited access to MM Tx are shown. In the R-phase, data included pt and disease characteristics at diagnosis, Tx response, comorbidities and treatment 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: 2 pts in first line (1L), 4 pts in second-line (2L) and 7 pts in third or higher lines. Analyses were descriptive.

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PB2010
SINGLE SHOT MEDIUM DOSE MELPHALAN IN RELAPSED MM PATIENTS: A RETROSPECTIVE, SINGLE CENTER EXPERIENCE
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Background: Multiple myeloma (MM) patients refractory to proteasome inhibitors, IMiDs or both, have an extremely poor prognosis. Moreover, they frequently fail to respond to further therapies, and represent a major challenge in everyday clinical practice.

Aims: With this in mind, we treated 12 patient with relapsed MM with a single shot of medium dose melphalan (60 mg/m2) between October 2010 and January 2016.

Methods: The median age was 72 years (range, 62 – 79) and the median time from initial diagnosis to melphalan treatment was 51 months (range, 24 - 144). Patients were heavily pretreated with a median number of 3 prior lines of therapy. All patients were refractory to the previous therapeutic regimens and had failed to respond or were refractory to regimens containing bortezomib. Seven patients (64%) had previously received at least one IMiD, 8 (67%) autologous stem cell transplantation (ASCT) and 1 allogeneic stem cell transplantation. The patients included in the series were not eligible for any clinical trial available at the institution. All patients gave informed consent.

Results: All patients had cytopenia (anemia, neutropenia and thrombocytopenia). We observed 3 cases of gastrointestinal toxicity (1 bleeding, 1 subcolliquion, 1 mucuscolitis), 1 WHO, 3 cases of clinically documented infection (1 Escherichia coli bacteremia, 1 fever of unknown origin, 1 erysipela) and 2 deep vein thrombosis. Response was assessed between six and eight weeks after melphalan therapy. Overall, 10 out of 12 patients had a response (1 complete response, 3 very good partial response, 2 partial response and 4 stable disease); only 2 had progressive disease. Median overall survival was 11 months (range, 2 - 37). 10 of 12 patients relapsed after a median time of 5 months (range: 2-12). Concerning two patients not relapsed, 1 patient died in partial response 8 months after therapy of other causes; 1 patient is still alive, in complete remission 18 months after melphalan. He underwent ASCT and maintenance with lenalidomide.

Summary/Conclusions: Many patients refractory to proteasome inhibitors and IMiDs are probably still sensitive to alkylating agents and could be rescued with medium dose melphalan. We suggest therefore melphalan as a “bridge” in everyday clinical practice.

PB2012
A FEASIBILITY-STUDY ON IMPLEMENTATION OF THE INTERNATIONAL MYELOMA WORKING GROUP RECOMMENDATIONS FOR MULTIPLE MYELOMA PATIENTS IN ROUTINE CLINICAL PRACTICE: A PERIPHERAL CENTER EXPERIENCE
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Background: Renal impairment (RI), defined as serum creatinine above upper normal limit or >2 mg/dl or a estimated glomerular filtration rate (eGFR) <60 ml/min/1,73m2, is one of the most common complications of MM, and it is associated with an increased risk of early death. The incidence of RI at MM diagnosis ranges from 20% to 50%, while its comparison occurred in 60% MM patients (pts). In this scenario tempestive diagnosis of RI in MM pts and exclusion of possible alternative causes of RI (like amyloidosis, diabetes or MIDD) are essential.

Aims: We applied a diagnostic algorithm obtained from the International Myeloma Working Group Recommendations in pts admitted to our department for RI (with known and unknown MM, or suspected cast nephropathy, CN), in order to investigate if this diagnostic workflow could positively impact on MM pt management.

Methods: We enrolled adult pts, known or unknown MM, admitted to our hospital for RI or suspected CN, with or without monoclonal component. Primarily, we performed complete blood analysis, with eGFR (CKD-EPI and MDRD methods), serum and urine electrolites, bicarbonatemia, serum and urine immunofixation, fraction 3 and 4 of complement, crioglobulinemia, HbA1c, arterial gas analysis, evaluation of urinary rate every 6 hours, daily urine collection, urine sediment analysis. We also collected anamnesis on eventual nephrotic or nephritic complaints therapy like ASA, FANS, clinical parameters and objectives signs of RI (edema, symptomatic disionia). On the second day of hospitalization we requested protein electrophoresis on serum and urine, chest X-ray, ultrasonography of abdomen, ecocardiography and electrocardiography. On the day three we evaluate the results of previous exams and we decided if nephrologic further exams or bioradiography (bone marrow in suspected unknown MM pts, renal in suspected CN pts, umbilical fat for amyloidosis). All analyses were daily and collegiately 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 knew MM with a de novo RI, 12 diabetes related RI, 3 amyloidosis, 16 other causes.

Summary/Conclusions: The implementation of the International Myeloma Working Group Recommendations in a routine clinical practice confirmed its feasibility and utility in the optimal workup of MM pts. We obtained diagnosis of RI within 4 days, both in known and in de novo MM pts, with a positive impact on reduced hospitalization, unnecessary dyalisis and steroids overtreatment.

PB2013

NOCARDIOSIS PROVOKED BY NOVEL AGENTS AT RELAPSED MULTIPLE MYELOMA: CASE SERIES
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Background: The proteasome inhibitors and immunomodulatory drugs which are used in MM treatment enhance the risk of infection by several mechanisms. Nocardial infections are rare in Turkey.

Aims: Here, we present three relapsed myeloma cases which developed nocardia pneumonia.

Methods: Case-1: 66 year old man, who has a history of autologous SCT 4 years ago and lenalidomide usage because of IgG kappa type myeloma, has been prescribed bortezomib for the relapse of the disease. He was immunocompromised not only because of the myeloma, and also because of the diabetes and renal failure without dialysis. He was admitted to the hospital because of the productive cough. His lymphocyte count was 1200/mm3 and flow-cytometric analysis couldn't be performed. Thorax CT showed 39x39x45 mm mass like lesion. Broncoscopic lavage examination showed branching bacillus via modified acid-fast and Gram stain. This typical morphological appearance was defined as Nocardia spp. Imipenem-cilastatin treatment started and control CT was performed after ten days and it showed regression of the infiltration. He was discharged with oral TMP/SMX antibiotherapy. Case-2: 71 year old woman, who has a history of two autologous SCT 12 and 5 years ago because of IgG kappa type myeloma; admitted to the hospital with productive cough during pomalidomide treatment. Her lymphocyte count was 2300/mm3 and flow-cytometric analysis showed CD5:%88 and CD 20:%1. HRCT showed a 7x6x6 cm sized mass like lesion with a cavity. Bronchial Gram positive bacillus (Nocardia sp.) was detected from broncoscopic specimen analysis, so imipenem-cilastatin therapy has been started. She responded well to therapy and was discharged with TMP/SMX antibiotherapy. Case-3: 72 year old man, who has a diagnosis of IgG kappa type myeloma and a history of autologous SCT 4 years ago following bortezomib treatment, relapsed 5 months ago. He has been admitted to the hospital with non-productive cough under the treatment of lenalidomide and dexamethasone. His lymphocyte count was 520/mm3. Flow-cytometric analysis couldn't be performed. Thorax CT showed 4 cm sized cavity and sputum microscopy showed acid-resistant branching bacillus thought to be consistent with nocardiosis. The imipenem/cilastatin and TMP/SMX treatment have begun and 12 days later, a control CT was performed and showed regression. He was discharged with oral TMP/SMX antibiotherapy.

Results: See Table 1 and Figure 1.

Table 1.

<table>
<thead>
<tr>
<th>General Status of Patients</th>
<th>Case-1</th>
<th>Case-2</th>
<th>Case-3</th>
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<td>2106</td>
<td>2309</td>
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<tr>
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<td>328</td>
<td>258</td>
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<tr>
<td>Prior Treatment</td>
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Summary/Conclusions: The proteasome inhibitors and immunomodulatory drugs which are used for the treatment of MM make T cell dysfunction and considering B cell dysfunction is also present because of the nature of the disease; this situation tends to provoke rare opportunistic infections such as nocardiosis. Thus, in these patients, it is significant to follow the lymphocyte count closely and to keep in mind that kind of rare microorganisms.

PB2014

LENALIDOMIDE IN PATIENTS WITH DIALYSIS-DEPENDENT END STAGE RENAL FAILURE (ESRF) AND MULTIPLE MYELOMA
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Background: Lenalidomide is an oral immunomodulatory medication with clinical efficacy in relapsed/refractory and treatment naive multiple myeloma (MM), Sjögren's syndrome, and lymphoma. Lenalidomide is eliminated predominantly unchanged by urinary excretion. Renal impairment is common in MM (15-40%) and approximately 10% of MM requires dialysis. However, there is a paucity of clinical safety data of Lenalidomide in ESRF. There is evidence that Lenalidomide can be safely used in patients with moderate and severe renal dysfunction with dose adjustment. However, published data in hemodialysis-dependent patients is limited to a handful of patients across small retrospective analyses and case reports. Patients with ESRF have generally been excluded from clinical trials investigating Lenalidomide. Phase III trials in the relapsed setting (MM-009, MM-010) excluded patients with a serum creatinine >221μmol/L. The FIRST trial (MM-020), investigating upfront use, excluded patients dependent on dialysis. There is no accepted clinical standard on the most appropriate dosing of Lenalidomide in dialysis. The manufacturer has provided guidelines, being 5mg daily, day 1-21, every 28 days (equivalent to 105mg per cycle). There is alternate well-cited pharmacological dosing that the more appropriate starting dose is likely 15mg, three times per week, given post-dialysis (equivalent to 135mg per cycle). Aims: To provide real-world evidence of an institutional experience of the use of Lenalidomide in dialysis-dependent MM.

Methods: We performed a retrospective audit of our in-centre experience with treating dialysis-dependent MM with Lenalidomide and included patients who completed at least one cycle of therapy. Patients were assessed for haematological toxicity, significant infective complications, thrombosis, disease response and progression-free survival. Best response was stratified by IMWG criteria. Patients' baseline characteristics, prior therapies, cytogenetics and FISH data were collected.

Results: We identified 5 patients treated between 2010 and 2017, aged between 54 to 73 years old. All patients had relapsed/refractory MM and dialysis dependent ESRF. The median number of prior therapies was two. One patient had (11,14) on FISH and died from progressive disease. Dose schedules are shown in the Table 1. Almost all patients experienced grade III-IV haematological toxicity and 60% had grade III-IV infection. There was a positive correlation between dose and toxicity, and furthermore there appeared to be an inverse relationship between age and tolerated dose. Haematological toxicities and infection were ameliorated by dose adjustment in most instances. There was no drug related mortality, however one patient died of progressive disease. Four of the five patients were prescribed aspirin thromboprophylaxis, with no proven thrombotic complications seen. Where possible to assess, the ORR was 75% (3/4), with 2 patients achieving a very good partial response (VGPR), 1 partial response and 1 progressive disease. The lowest starting dose in this cohort was 10mg twice/week and the maximum dose was 25 mg three times/week.

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Summary/Conclusions: Our experience builds on the emerging evidence that reduced dose of Lenalidomide can be safely prescribed for dialysis-dependent MM with clinical efficacy, our cohort most patients on days of dialysis only. There was significant variation of dose-related tolerability between patients. However, toxicity was manageable with diligent monitoring and dose adjustment.
TU2015

STUDY USE OF 18-F FDG PET / CT SCANNING INTO THE FIRST FOLLOW-UP OF PATIENTS WITH MULTIPLE MYELOMA AND ASSOCIATION WITH BIOCHEMICAL RESPONSE


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Background: Positron computed tomography (PET / CT) with 18F fluoro-deoxyglucose-labeled glucose (FDG) is a reliable technique with high sensitivity and specificity for assessing skeletal involvement and recent studies propose it as a method for predicting treatment response in multiple myeloma. Conventionally, the response is measurable by the monoclonal component in both serum and urine and Minimal residual disease (MRD) by flow cytometry has been established as a mandatory tool. The studies are aimed at combining the measurement of paraprotein with imaging tests that help to promptly define response or failure to the treatment.

Aims: The primary endpoint was the correlation of the biochemical response with the FDG PET/CT in a second evaluation after first line treatment. The secondary endpoint was the correlation between MRD and second FDF PET/CT.

Methods: We included in this retrospective and observational study at University Hospital of Vall d’Hebron, all patients with newly MM and PET/CT before to start a first line treatment and a second PET/CT when completing treatment. PET/CT were analyzed by the department of Nuclear Medicine with experience to grade the lesions in MM, were evaluated and categorized into positive or negative according to the criteria proposed by Zamagni, et al. The biochemical response was defined according to the standard IMWG response criteria. The biochemical response was defined according to the standard IMWG response criteria.

Results: Eighteen patients (8 males and 10 females) with untreated MM entered, seven patients were classified with ISS III, fifteen had a good performance status, none presented renal lesion, only 16% had hypercalcemia and 66% showed immunorepress. Ten patients were IgG isotype, six were classified as light chains myeloma and two patients were oligosecretors. Seventeen patients had bone marrow infiltration with a median of 42% plasmatic cells. Two patients had 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 was positive in patients with complete response. We found discordant data in two patients with oligosecreatory myeloma. No correlation was shown between PET/CT and flow MRD. Are necessary more long term studies that include greater number of patients to confirm that the PET/CT negative is an image technique that could be a tool to follow up patients after the first line treatment added to the evaluation of the biochemical response.

PB2016

MULTIPLE MYELOMA IN BORNEO SARAWAK: A DEVELOPING WORLD’S EXPERIENCE

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1Hematology Unit, Medical Department, Sarawak General Hospital, Kuching, Malaysia

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 hematologist/transpose 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 2015 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 Bidayu 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 overall survival was comparatively lower to our counterparts. Limitation 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 RADIOLOGICAL INVESTIGATIONS FOR SUSPECTED CASES OF PLASMA CELL DYSCRASIAS/MYELOMA IN THE ALTNAGELVIN AREA HOSPITAL

G. Baker1,*, N. Cunningham1

1Haematology, Western trust, Londonderry, United Kingdom

Background: The updated NICE guidelines for diagnosis and management of myeloma (2016) suggests whole-body MRI as first-line imaging for people with suspected myeloma and consideration of MRI/CT/PET in newly diagnosed myeloma to assess for bone disease or EM plasmacytoma.

Aims: Our aims were to ascertain; 1) Our current practice regarding radiological investigation for myeloma (2) Whether additional diagnostic information was gained using CT/MRI imaging (3) Since its release, is the trust compliant with the NICE guidance (4) The estimated cost of meeting the current NICE guidance

Methods: This retrospective and prospective audit included all patients having a skeletal survey performed for suspected multiple myeloma within the Alt-nagelvin Area Hospital (AAH). Retrospectively from 10/2/15 until 9/2/16 data was collected using the advanced search feature of the Spectra ID57 PACS system. The ‘Reason for examination’ for each study was then analysed and those ordered for reasons other than suspected myeloma were excluded. Each case was analysed individually and any follow up MRI/CT/NMB imaging performed in the 6 month period following the skeletal survey were included in the data collection. The same information was gathered prospectively from 10/2/16-30/5/16 following the NICE guidance. 54 skeletal surveys where performed for suspected/re staging myeloma pre guidance.

Results: The indications for requesting imaging is shown in Table 1A. No WB MRI/CT was performed in this period. 26% patients had new lytic lesions on skeletal survey. 23 patients had further imaging in the form of MRI or CT following skeletal surveys. All the positive MRI findings offered additional diagnostic information - including examples of missed multiple spinal deposits. The results of imaging are summarised in Table 1B. The false negative rate for skeletal surveys was 39% and the false positive rate was 22%. Following NICE guidance publication 23 patients had skeletal surveys performed for suspicion of myeloma between 10/2/16 and 30/5/16. The indications are summarised in Table 1C. No WB imaging was performed. 5 patients had positive skeletal surveys. 6 patients had subsequent CT/ MRI imaging. A skeletal survey was reported normal with a subsequent MRI showing multiple spinal deposits. The imaging results are summarised in Table 1D.

Table 1A.

Table 1.

Table 1C. No WB imaging was performed. 5 patients had positive skeletal surveys. 6 patients had subsequent CT/ MRI imaging. A skeletal survey was reported normal with a subsequent MRI showing multiple spinal deposits. The imaging results are summarised in Table 1D.
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 imagining. At present failure to comply with NICE guidance will lead to delayed diagnosis of myeloma in certain patients and potential patient harm. Therefore I offer a business and health improvement case for the Western Trust to instigate WB-MRI imaging for all suspected myelomatous bony disease.

**PB2018**

**TONI DEBRE FANCONI SYNDROME DURING MYELOMA, ABOUT 8 CASES**

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**Background:** The cast nephropathy with cylinders is the most frequent renal complication of the myeloma, which results from a catabolism of the light chains by the tubular cells and can lead a tubular chronic suffering showing itself by a syndrome of acquired Toni-Debré-Fanconi marked by a glycosuria, a phosphaturia, an aminoaciduria, a sometimes severe and sometimes revealing hypokalemia.

**Aims:** We reporting some observations informed by Multiple Myeloma complicated with a Fanconi syndrome.

**Methods:** From January 2000 till December 2010: 78 cases of Multiple Myeloma were brought together, whose circumstance of discovery 22 cases with renal failure, it’s was a evolutes complications in 12 cases; and in 10 cases it’s discovered at diagnosis. The renal achievement is dominated by Tubule disease in 11 cases, Randall syndrome 8 cases, and Nephrotic syndrome in 3 cases. The tubule disease of Fanconi is suspected at only 8 patients: in front of the presence of a glycosuria (without associated diabetes) and a frank proteinuria in the majority of the cases, with a hypophosphatemia and a flicky 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 oesopage pains and the muscular cramps dominate the clinical presentation with constant diffuse demineralization in the radiology. - The patients were classified (according to the Salmon-Durie classification): IIIB (3 cases) and IIIIB (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 strategies double: - Symptomatic: alkaline hydration, correction of the metabolic disorders and sometimes the renal extra pure (indicated in 3cases). - Specific: chemotherapies VAD cases, a patient died by cardio-vascular complication. Under treatment the recovery of the renal function is obtained in 3 cases, to the 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 autopsie series. It is necessary to think to it in front of any renal achievement in myeloma of kappa light chain with renal glycosuria, a generalized aminoaciduria and a hypophosphatemia resulting respectively from a defect of the transport of the glucose, from amino acids and from phosphates by the renal proximal tubule. To improve the osseous and renal appearances, it is necessary to realize a calcic supplementation, phosphorous and by the vitamin D active, as well as the correction of the 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 plasma exposure was established as well tolerated and in the phase II part the study focused the efficacy and toxicity in the subgroup treated with maximum planned dose. The ASPIRE trial showed superior response rates and progression free survival for carfilzomib-lenalidomide-dexamethasone compared with lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients.

**Aims:** The aims is explore the efficacy and tolerability of carfilzomib-lenalidomide-dexamethasone in relapsed/refractory Multiple Myeloma patients in real life.

**Methods:** All patients received carfilzomib 20/27 mg/m2 days 1,2,8,9,15 and 16; lenalidomide 25 mg days 1-21 and dexamethasone 20 mg days 1,2,8,9,15, 16, 22 and 23, according to post approval access protocol. After 2, 4, 6 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 1-4); 9 (60%) received previous autologous transplantation while 1 (6%) allogeneic; 11 (73%) 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>62 (28-79)</th>
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<tbody>
<tr>
<td>MULTIPLE MYELOMA, n (%)</td>
<td></td>
</tr>
<tr>
<td>RELAPSED</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>REFRACTORY</td>
<td></td>
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<tr>
<td>MULTIPLE MYELOMA, subgroup, n (%)</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>6 (40)</td>
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<tr>
<td>IgA</td>
<td>2 (13)</td>
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<tr>
<td>MICROMOLECULAR</td>
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<tr>
<td>STAGING, (N)</td>
<td></td>
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<tr>
<td>I</td>
<td>II</td>
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<tr>
<td>3 (20)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>1 (80)</td>
<td>1 (60)</td>
</tr>
<tr>
<td>MEDIAN TIME FROM DIAGNOSIS TO KD, months (range)</td>
<td>46 (12-92)</td>
</tr>
<tr>
<td>MEDIAN OF PRIOR THERAPY, days (range)</td>
<td>3 (1-6)</td>
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<tr>
<td>PRIOR TRANSPLANT, n (%)</td>
<td></td>
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<tr>
<td>9 (60)</td>
<td>1 (6)</td>
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<tr>
<td>AUTOCLOGUES</td>
<td></td>
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<tr>
<td>ALLOGENIC</td>
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<tr>
<td>PRIOR THERAPY, %</td>
<td></td>
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<tr>
<td>LENALIDOMIDE</td>
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<tr>
<td>BORTEZOMIB</td>
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<tr>
<td>POMALIDOMIDE</td>
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</tbody>
</table>

**Figure 1.** Summary/Conclusions: Carfilzomib-lenalidomide-dexamethasone is a powerful and efficacy association in relapsed/refractory 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.

Madrid, Spain, June 22 – 25, 2017

haematologica | 2017; 102(s2) | 803
Results: and IMIDs, and 33% had also received radiotherapy. 57% of them had under-
and in particular one del13q14 1qgain, one del 13q14 and one t(11;14). 86%

ISS was equally distributed, and cytogenetic was evaluable in 8 patients,
on days 1,8,15 and 22, every 28 days) for a median treatment cycles of 2 (r 1-
thereafter; Lenalidomide 25 mg on days 1 through 21; Dexamethasone 40 mg
zomib starting dose 20 mg/sqm on days 1,2 of cycle 1, target dose 27 mg/sqm
thereafter: Carfilzomib was well tolerated, with grade 2 anemia in 28% of
patients, without necessity blood transfusions; 5% grade 1 and 9.5% grade
3 neutropenia (no ospedalization 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 Hypertension in 24% of
patients, without necessity of blood transfusions; 5% grade 1 and 9.5% grade
3 arrhythmias in 5% of patients; grade 2 dyspnea in 5% of
patients; grade 1 fatigue in 9.5% of patients. According to IMWG criteria, after
a median follow-up of 3 months (r.1-13), ORR was 66,7% (14/21 :  8 VGPR, 6
PR) with 3 progressive diseases and 2 patients in stable disease, which can
be considered as an impressive result in this subset of rrMM patients. In par-
ticular, for 1 patient, KRD was, after having achieved at least a PR, a bridge to
second auSCT. Median time to response was 2 months (r.1-4), median OS from
diagnosis was 47 months (9-170 range), median OS from start of Carfil-
zomib was 3 months (range 1-13).

Table 1.

Summary/Conclusions: KRD has shown significant efficacy in a particularly
selected setting of patients, relapsed and refractory to all available therapeutic
resources, and, in particular cases, it could be considered as a bridge to a sec-
ond autologous or allogenic SCT.
Background: The treatment of patients with multiple myeloma (MM) has dramatically changed over the past decade due in part to the development of new agents and myeloma-specific targets. Nowadays, new effective treatments exist for patients with RRMM not responding to bortezomib and lenalidomide. Pomalidomide alone has shown limited efficacy in patients with RRMM, but synergistic effects have been noted when combined with dexamethasone.

Aims: To show our experience with the use of 28-day cycles of pomalidomide (4 mg/day on days 1–21, orally) plus low-dose dexamethasone (40 mg/day weekly, orally) (Pom/dex) in RRMM.

Methods: This is a retrospective study performed between May 2014 and January 2017 in the Hospital of Guadalajara (Spain). Eight patients (3M, 5F), with a median age of 67 years (range, 40-81), diagnosed with MM and with 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 (IGR: 56.6-80.25), 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 (IGR: 48.25-140.25), 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 (IGR: 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.

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 hypereosinophilia (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 idiopathic neutrophilic dermatosis (CEL-NOS) patients negative for PDGFRαβ abnormalities (Barba J et al., Oncotarget. 2014;5:4865-70).

Consequently it has been suggested that HES patients should be screened for KIT M541L, as positive cases may benefit from imatinib treatment.

Aims: We aimed to (i) compare the KIT M541L allele frequency between patients referred for investigation of HE and normal healthy controls and (ii) investigate the variant allele frequency (vaf) to determine if KIT M541L mutations may be acquired somatically and (iii) investigate the KIT M541L status in cases negative for PDGFRαβ abnormalities who responded to imatinib.

Methods: We screened healthy controls (n=214) and patients referred for investigation of FIP1L1-PDGFRα negative HE (n=220) for KIT M541L using an amplification refractory mutation system (ARMS) PCR designed to amplify allele specific products of different sizes, and able to detect KIT M541L down to 5% vaf.

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 (0.06% versus 0.09%; P=0.91). Of the 42 KIT M541L heterozygous HE cases, 40 had sufficient DNA for analysis by ddPCR. The mean allele burden was 50.4% (range 48.3%-56.0%), consistent with all instances being constitutional. None of the three imatinib responders tested positive for KIT M541L prior to treatment.

Summary/Conclusions: Whilst we cannot exclude the possibility that KIT M541L may be acquired somatically in very rare cases, we conclude that there is no clinical value in screening for this variant on a routine basis for patients with HE or HES.

PB2024

MUTATIONS OF THE JAK2 GENE AND CYTIGENIC 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, JAK2, PDGFRαβ and PDGFRβ 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.

Aims: 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 mean, or a value greater than 11,000/μL. Those patients who satisfied the following criteria were included in the study: leukocytosis (predominantly neutrophilic); the absence of apparent causes of leukocytosis; and documentation of the leukocytosis over a prolonged period

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.
of time. The period of observation was 1 year or longer in most patients. Sixteen patients with idiopathic leukocytosis were analyzed in this 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. Genomic 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 CEPPA 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 transcription polymerase chain reaction (RT-PCR) were performed between the ETV6 and ABL1 genes in 10 patients. BCR/ABL1 gene was analyzed by RT-PCR or fluorescence in situ hybridization in 8 patients. The current study was conducted within the guidelines and with the approval of the Ethics Committee.

Results: JAK2 V617F mutations were found in one of the 16 idiopathic leukocytosis patients. No mutations were found in the other genes in the 16 idiopathic leukocytosis patients. ETV6-ABL1 fusion gene was detected in one of the 10 patients. No BCR/ABL1 fusion gene was detected in the 8 patients. One 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). ETV6-ABL1 fusion gene was detected in this patient. Another patient with normal karyotype progressed to blast crisis of Ph-CML, identified 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.
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 viral oncogene (MPL), and calreticulin (CALR), have been identified in most patients with BCR-Ab1 negative MPNs. JAK2 mutations are present virtually all patients with polycythemia Vera and 50-60% of pmf and Essential Thrombocythemia (ET). Recently, mutations in CALR gene were found in 50-80% of JAK2 and MPL mutation negative ET and pmf 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 (pmf) patients.

Methods: Bone marrow biopsy samples from 32 patients previously diagnosed as primary myelofibrosis with known JAK V617F mutation status were obtained from archives of Marmara University Pathology Laboratory. Bone marrow samples of two patients were already known as CALR mutated by PCR analysis. Bone marrow samples of three JAK2 wild type and CALR mutated ET, two JAK2 wild type, CALR mutated pmf patients and two CALR wild type ET patients were used as positive and negative control tissues for CALR immunohistochemistry. Immunohistochemistry: 4-µm unstained sections of each bone marrow biopsy specimens were cut onto electrostatically charged glass slides. Immunohistochemistry was performed on an automated immunostainer (Ventana BenchMark Ultra; Ventana Medical Systems, Inc). CALR antibody (clone CAL2, Dianova, Germany) staining used a 1:100 dilution. Any cytoplasmic staining of the cells with CAL2 antibody was considered positive immunostaining.

Results: We studied 32 bone marrow specimens of primary myelofibrosis with 15 (47%) of them having JAK2 V617F mutation and 17 (53%) of them lacking JAK2 V617F mutation. CALR immunoreactivity was seen in 8 (25%) of all pmf patients. CALR (MPL) and cal was seen in 8 (47%) of patients with PMF myelofibrosis who are negative for JAK2V617F mutation. CALR immunoreactivity was not seen in patients with PMF myelofibrosis who are positive for JAK2V617F mutation. CALR immunoreactivity was seen in 3 (100%) of patients with ET and 2 (100%) of patients with known CALR mutation. CALR immunoreactivity was seen in patients with CALR wild type ET patients. We observed that CAL2 immunostaining was seen mainly in the cytoplasm of the small and large megakaryocytes, and atypical megakaryocytes as found in fibrotic pmf. Pale immunostaining was seen in myeloid and erytoid cell precursors. This immunostain also stained some small cells appearing as micromegakaryocytes.

Summary/Conclusions: An immunohistochemical stain easily detects the CALR mutation by staining of megakaryocytes in formalin-fixed bone marrow biopsy specimens. This method would be a easy, rapid, and cost effective way to detect CALR mutations in daily routine hematopathology biopsy evaluation of the myeloproliferative patients.

PB2028

THE HIF1A/2A MRNA INDEX HAS A SIMILAR TREND AS THE CHANGES OF EXPRESSION MRNA CALR AND MDR1 GENES IN WHOLE BLOOD SAMPLES OF PATIENTS WITH JAK2 V617F POSITIVE MPN

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Background: Various groups have reported that isofoms of hypoxia-inducible transcription factor 1a (HIF-1a) and 2a (HIF-2a) regulate both overlapping and distinct target genes. HIF-1a and HIF-2a have been shown to play opposite roles in the regulation of macrophage function [Takeda N. e.a, 2010]. HIF-index incorporated as a strong prognostic biomarker of renal cell cancer [Szendrői A. e.a., 2016]. Only HIF1a was known as regulator expression of multidrug resistance gene (MDR1) and response to chemotherapy [Comerford K.M. e.a., 2002]. New data have shown exclusive role of HIF-2α in regulates the proliferation and activity gene (MDR1) and response to chemotherapy [Xu Q.Q. e.a., 2016]. Only HIF1a was known as regulator expression of multidrug resistance (MDR1) and response to chemotherapy [Comerford K.M. e.a., 2002]. New data have shown exclusive role of HIF-2α in regulates the proliferation and activity gene (MDR1) and response to chemotherapy [Xu Q.Q. e.a., 2016]. No any information is available on the role of HIF-1α and HIF-2α in the transcription of MDR1 and CALR.

Aims: Investigate the mRNA expression levels of HIF-1α and HIF-2α, MDR1 and CALR genes in whole blood samples of patients with JAK2 V617F positive MPN.

Methods: Real-time PCR was performed to detect HIF1a, HIF2a, MDR1 and CALR mRNA transcripts levels in white blood cells 14 healthy volunteers (median age 22 years, range 21-58 years, 57% males) and 11 (median age 44 years, range 20-77 years, 45% males) patients with JAK2 V617F-positive MPN, median of alelic burden is 36%, range 9-87%. Venous blood was collected in tube with RNAse inhibitor. Total RNA was isolated using “RIBO-zol-d” (Aplisens) and were transcribed using “Reverta-L” (Aplisens). PCR was optimized for the thermocycler CFX96 (Bio-Rad). The results were calculated using the delta Ct method in the software package of “R”. The threshold cycles (Ct) genes and housekeeping genes (TPB, GUS, ABL) determined using Cy0 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 HIF2a not changed and for HIF1a 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 mRNAAs (r=-0.61 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.
DETECTION OF THE MUTATIONS IN GENES JAK2 AND MPL IN THE DIAGNOSIS OF CHRONIC MYELOPROLIFERATIVE DISORDERs

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Background: Chronic myeloproliferative diseases is a group of clonal Ph-negative hematological diseases, which include erythremia (polycythemia Vera, PI), chronic megakaryocytic leukemia (essential thrombocythemia, ET) and subleukemic myelosclerosis (primary myelofibrosis, PMF, chronic idiopathic myelofibrosis). The origin of these diseases is linked to transformation of hematopoietic stem cells, the result is the excessive production of mature cells of erythroid, granulocytic and megakaryocyte shoots with relatively long course of the disease. The frequency of occurrence of mutation V617F of gene JAK2 exon 12 and MPL gene varies in different literature.

Aims: Determination of the frequency of occurrence of mutations in genes JAK2 and MPL and identifying the importance of the verification of these diseases.

Methods: The study included 350 patients with chronic myeloproliferative diseases — with polycythemia Vera 150 patients, with essential 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 "AmpliPrep RIBO-prep" (OOO Interlabservice, Russia). The concentration and purity of isolated DNA was determined by Nano Drop 2000 instrument (USA). Detection of gene mutation JAK2V617F and MPL gene was carried out by standard polymerase chain reaction on a thermal cycler 2720 "Applied Biosystems" (USA), using a set of "Litech" (Moscow).

Results: The result of the research showed that the incidence of the V617F mutation in JAK2 was varying in patients depending on the type of disease. In polycythemia Vera the mutation V617F in the JAK2 gene was identified in 147 patients (98.3%), with essential 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, wich examined with the purpose of differential diagnosis with Ph(-) chronic myeloproliferative diseases, V617F in JAK2 was detected in 6 (8.6%), which allowed to confirm Ph(-) Chronic myeloproliferative diseases. A mutation in exon 12 of the JAK2 gene was detected in 2 of 33 (2.9%) of those surveyed V617FJAK2-negative patients exclusively diagnosed with polycythemia Vera. The MPLV651L mutation gene was detected in polycythemia Vera and chronic idiopathic myelofibrosis 2.2% (1 of 41) and 2% (1 of 52) of patients.

Summary/Conclusions: Thus established, our data confirm that mutations in the genes JAK2 and MPL are highly specific diagnostic markers in patients with Ph-negative chronic myeloproliferative diseases.

ASSOCIATION OF MYELOPROLIFERATIVE NEOPLASM AND LYMPHOPROLIFERATIVE DISORDER IN 3 PATIENTS

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Background: Lymphoproliferative disorders (LPD) and myeloproliferative neoplasms (MPN) are two very different sets of hematological pathologies. However, several studies have shown that the risk for LPD onset in patients with MPN is higher than in the general population (1)(2). No single LPD seems to be more at cause and all MPN are likely to present the onset of an associated LPD.

Aims: We present 3 cases diagnosed in the Department of Hematology, « Groupement Hospitalier Est », Lyon, France, of patients bearing an association of MPN and LPD: an essential thrombocythemia (ET) with myeloma, ET with marginal zone lymphoma and a chronic myeloid leukaemia with chronic lymphoid leukaemia.

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. 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 nor cytopenia. Medullar blood was diluted but showed slightly atypical plasmocytes remaining under 10%.Myeloma was diagnosed anyway and the patient received 5 cures of 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 leukaemia, 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 situations observed in cases of combined MPN/LPD pathologies. MPN with secondary onset of LPD are most frequently encountered, as was the case with patients 1 and 2. Cases of preexisting LPD and late onset MPN are rare (1), and cases of simultaneous discovery of both pathologies even more so (3). Several hypotheses have been formulated to explain the frequency of onset of these pathological associations: genomic instability due to JAK2 protein kinase activation, or due to BCR-ABL mutation, or exposure to cytotoxic chemotherapy or radiations (3).
Myeloproliferative neoplasms - Clinical

PB2032

CLINICAL AND ANALYTICAL DIFFERENCES BETWEEN CALR TYPE-1 AND CALR TYPE-2 MUTATION IN PATIENTS WITH ESSENTIAL THROMBOCYTHESIA 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 mutation was found in negative patients. There are two main variants of CALR mutation: type 1 (a 52-bp deletion) and type 2 (a 5-bp insertion).

Aims: To compare clinical and analytical data of ET and PMF patients with CALR type-1 vs CALR type-2 mutation.

Methods: We performed a single center study on 471 patients: 87 PMF and 384 ET. The JAK2V617F mutation was analyzed in DNA from peripheral blood leukocytes by PCR ARMS method. In all JAK2V617F negative patients detection of CALR mutation was performed by fragment length analysis and the results were confirmed by sequencing. Statistical data analysis was performed using the Statistica 12.5 software for Windows.

Results: From 384 ET patients 254 were JAK2V617F positive (66%), 80 were CALR positive (21%) and 51 were JAK2V617F and CALR negative (13%). From CALR positive patients: 36 (51%) had type-1, 34 (45%) type-2 mutation, and 10 (12%) type-3 mutation. From 87 PMF patients 56 were JAK2V617F positive (64%), 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 (1067 vs 800 G/L, p<0.001) but with no significant differences in frequency of thrombosis. In ET, CALR mutation was associated with increased odds of myelofibrotic transformation (odds ratio [OR]=2.61; 95% CI: 1.28 - 5.34; p=0.009) comparing with JAK2V617F positive patients. Patients ET CALR type-1 had higher leukocyte counts than ET CALR type-2 mutation (9.6 vs 7.3 G/L, p= 0.008), but we did not find significant differences in hemoglobin, platelet counts, frequency of thrombosis or myelofibrotic transformation. Within PMF, no significant differences were observed. Moreover in PMF, there was no significant differences between the JAK2V617F, CALR type-1 and type-2 mutation status according to the International Prognostic Score System (IPSS).

Conclusion: The JAK2V617F 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.

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.

<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>
</tr>
<tr>
<td>5</td>
<td>1 (0.92)</td>
</tr>
</tbody>
</table>

Table 2. Drugs used in patients with ET.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxyurea</td>
<td>99</td>
</tr>
<tr>
<td>Anagrelide</td>
<td>31</td>
</tr>
<tr>
<td>Interferon</td>
<td>10</td>
</tr>
<tr>
<td>Busulfan</td>
<td>4</td>
</tr>
<tr>
<td>Melphalan</td>
<td>1</td>
</tr>
<tr>
<td>Danazol</td>
<td>2</td>
</tr>
</tbody>
</table>

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 (triage-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: Outpatients’ 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 JAK2V617F 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+), 18/240 (7.5%) – MPL-positive (MPL+). 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 CALR+ – 18.2% (2/11) pts and no cases of thrombosis were detected in CALR2+ and MPL+ subgroups (p>0.001). There were significant statistical differences in

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Madrid, Spain, June 22 – 25, 2017
median platelet count as follows: 742х10⁹/l (thrombosis+) and 937х10⁹/l (hemorrhage+) (p=0.03). No significant statistical differences in median age and leukocyte count (p=0.75 and p=0.47) were detected. There were more than a half pts older than 60 years in groups NC (51%) and thrombosis+ (59%) and in group hemorrhage+ only 36% (p<0.001). Cardiovascular risk factors were reported in 24% pts (NC), 69% pts (thrombosis+) and 36% pts (hemorrhage+) (p<0.001). There were no significant statistical differences in follows risk factors as thrombosis+ >100x10⁹/l and leukocytosis+ >11x10⁹/l (p=0.85 and p=0.72). No significant differences in OS among groups NC, thrombosis+ and hemorrhage+ (p=0.12) were found (Figure 1).

**Figure 1.**

**Summary/Conclusions:** Leukocytosis >11x10⁹/l and thrombocytosis >100x10⁹/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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**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 2014). However, due to the high cost of sequencing, developing a twostage algorithm for detect mutations in JAK2 exon 12 using inexpensive screen-sequencing 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 N1-5 patient mutations was determined by pyrosequencing: N542-E543del (c.1624_1626delAAATGAA); I540-E543delinsGK (c.1619_1627 TCA-gAAATGK – c.1622_1627delGAAATG) and p. H538K539L (c.1612_1616CACAA>TT). These mutations have been already described. Main characteristics of 5 patients with JAK2 exon 12-mutated PV are reported in Table 1. The PV diagnosis of N1, 2, 3 and 5 patients was confirmed by bone marrow trephine biopsies histological examination. All five patients with JAK2 exon 12-mutated PV have an increased number of red blood cells, along with an accompanying increase in the concentration of hemoglobin and hematocrit level in the peripheral blood. Some of them had increase number of leukocytes and platelets in the disease dynamics. N1-4 patients were treated phlebotomy only and did not received any cytoreductive treatment to date. Patient N5 received hydroxyurea (HU). Importantly, two out five patients with JAK2 exon 12-mutated PV also have a mutation JAK2V617 (c.1141A>C). JAK2 exon 12 allele burden in sample from N1 patient is significantly increased in the disease dynamics.

**Table 1.**

**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**

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**Background:** In the West Midlands region of the UK, all patients with a suspected myeloproliferative neoplasm (MPN) have access to quantitative analysis...
of JAK2 V617F by droplet digital PCR as standard of care. The British Com-
mittee for Standards in Haematology recommends that suspected MPN cases
have investigation of JAK2 exon 12, CALR and MPL genes if JAK2 V617F is
negative.
Aims: The aim of the project was to improve the MPN service by substituting
sequential analysis of individual target regions within the JAK2, CALR and MPL
genes with a single assay, and to increase the number of genes available for
analysis.

Methods: A commercial next generation sequencing (NGS) gene panel (Oxford
Gene Technology, SureSeq Myeloid Panel), coupled with the Illumina MiSeq
platform was validated and implemented. The gene panel utilises hybridisation
based enrichment technology and consists of 25 MPN-related genes. During
the validation stage the following were enriched and analysed: 29 positive con-
trol samples with 30 known pathogenic variants, 30 negative control samples
without known pathogenic variants in the JAK2, CALR and MPL genes, and 24
MPN samples of unknown mutational status. Thus so far over 200 clinical sam-
ple s have been analysed and reported since the service was introduced in
October 2016.

Results: The panel has successfully identified: a large range of known patho-
genic 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
precedes 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 pro-
cedure also explored coverage and limits of sensitivity, potential chemistry spe-
cific artefacts and identified common polymorphisms for all 25 genes.

Summary/Conclusions: The panel has replaced the current sequential analysis
of CALR, MPL and JAK2 exon 12 in JAK2 V617F negative patients and reduced
turn-around-times with increased accuracy and sensitivity compared to Sanger
sequencing and fragment analysis. Our current clinical service operates on a two
tier system whereby clinicians can request analysis of the full 25 gene panel or
a 4 gene subset (JAK2, CALR, MPL, CBL as an in silico analysis).

PB2037
IN JAK2V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS, BLEEDING RISK CORRELATES WITH ALLELE BURDEN
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Background: Myeloproliferative neoplasms (MPN) are characterized by the
presence of JAK2V617F mutation that is almost invariably associated with poly-
cythemia vera (PV), but also occurs in the majority of patients with essential
thrombocythemia (ET) or primary myelofibrosis (PMF). JAK2V617F-positive
patients display different laboratory and clinical features from JAK2 wild-type
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 mor-
tality in MPN are thrombotic and hemorrhagic complications, albeit bleedings
are less frequent than thrombosis and mostly represented by minor hemor-
rhages (eg, epistaxis, menorrhagia and gingival hemorrhage). The impact of differ-
ent 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 25-50%, 3rd quartile 51-75% and 4th quartile 76-100%). Nominal
variables were compared with X2 test or Fisher’s exact where indicated. Sur-
vival has been evaluated only for groups with different prevalence of events
during follow-up and were calculated with the Kaplan Meier method and com-
pared using the Log Rank test.

Results: Three patients (1.2%) bleed at diagnosis (1 major and 2 minor hemor-
rhages) while 27 (11.8%) suffered for hemorrhages during follow-up (10 major
and 17 minor). Prevalence of hemorrhages results higher in 4th quartile com-
pared 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
pat/s for 1st quartile, 0.65/100 pat/s for 2nd quartile, 1.26/100 pat/s for 3rd
quartile and 3.23/100 pat/s for 4th quartile with a IRR of 5 and of 4.6 for the
4th quartile versus 2nd and 1st one. No statistically significant dif-
ference was observed in the use of anti-thrombotic drugs among patients of the
different quartiles.

Summary/Conclusions: Risk factors for hemorrhage in MPN are not well
defined, and there is no risk estimation model for this outcome. Acquired von
Willebrand disease, entity of platelet increased count and aspirin use have been
implicated in bleeding occurrence. Previous reports fail to demonstrate a
relation 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 hemor-
rhagic events.

PB2038
JAK2 ALLELE BURDEN IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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 gia, 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 an independent risk factor
for venous thromboembolic complications. New treatment with Ruxolitinib may
decrease JAK-AB but at the present, it is not clear the clinical advantage of
JAK-AB.

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 asso-
ciation with standard demographic, clinical and laboratory parameters with par-
ticular interest for thrombotic risk.

Methods: Peripheral blood samples from patients with Ph-negative cMPNs were
collected, DNA from leucocytes was analysed for JAK-2 (V617F) gene mutation
with amplification-refractory mutation system (ARMs) PCR, subsequently a real-
time quantitative polymerase chain reaction (qRT-PCR) for JAK2V617F allele
burden measurement was applied. A multivariate analysis was than performed
to evaluate any association of AB with demographic and clinical data.

Results: One hundred and twelve patients with Philadelphia negative cMPNs
were investigated: 52 females with a median age at diagnosis of 69 years (age
range: 18-95), 60 males with a median age of 68 years (age range: 18-
82 years). Thirty-four patients had Essential Thrombocythemia (ET), fifty-two
had Polycythemia Vera (PV) and twenty-six had primary myelofibrosis (PMF).
JAK-2 AB of patients with an age of <69 years and ≥69 years, was respectively
evaluated. Patients older than 69 years showed a significantly higher JAK2-AB
. JAK-AB was significantly reduced in ET, when compared to PV and PMF.
No correlation was found between median values of allele burden and IPSS and
DISS scores. In patients with PV (n=52), a significant correlation was observed
between allele burden and WHO2008 scoring system. No significant correlation
was found between allele burden and thrombotic risk according to IPSET-t and
IPSET-ET for PV and ET, respectively. Patients with a previous history of throm-
botic events had the highest JAK2-AB. In PMF, a positive correlation between JAK-
AB and grading of fibrosis was found only for the highest grades (MFIII 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 sys-
tem in all cases.

PB2039
COMPARISON OF CLINICAL AND LABORATORY DATA, INCLUDING JAK-2 46/1 HAPLOTYPE, BETWEEN PATIENTS WITH IDIOPATHIC ERYTHROCYTOSIS (IE) AND POLYCYTHEMIA VERA (PV)
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Background: Idiopathic erythrocytosis (IE) is a relatively rare finding charac-
terized 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 ana-
lized, nominal variables were compared with X2 test and continuous variables
with the Mann-Whitney test.

Results: Overall, 40 patients with IE and 93 patients with PV were included in
the current analysis (Table 1). Splenomegaly and itch were reported only in
patients with IE. History of thrombosis and cardiovascular events was pos-
itive 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.
Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>PV</th>
<th>IE</th>
<th>p</th>
</tr>
</thead>
<tbody>
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<td>48</td>
<td>0.001</td>
</tr>
<tr>
<td>Female 2</td>
<td>52</td>
<td>49</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Results: The fusion genes of rearrangements of PDGFRB and B genes were detected by RT-PCR. Immunohistochemistry analysis was carried out by flow cytometry. Most of all cases were treated with IM and followed up.

Summary/Conclusions: In the current study, we highlight 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 ASPRIN IN PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA

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Background: The essential thrombocytemia (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 anagrelide 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. 84-160 s) (240±40 s).

Summary/Conclusions: These findings suggest that in ET patients the daily low-dose ASA represents an optimal dosage strategy and that PFA test may be an useful tool to distinguish between the ASA "normal-responder" and "low-responder" ET patient.

**PB2041**

CLINICAL AND EXPERIMENTAL CHARACTERISTICS OF MYELOID/LYMPHOID NEOPLASMS DISPLAYING PDGFRB OR PDGFRB 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 PDGFRB, PDGFRB are classified in Myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRB, PDGFRB, or FGFR1, or with PMCL-JAK2. It is a rare event that patients presented rearrangements of PDGFRB. In the past decade, the dose of TKI to cases with PDGFRB and B abnormal was inconclusive.

Aims: The goal of the study was to assess the clinical and experimental characteristics and observe the response of Imatinib(IM) therapy of Myeloid/lymphoid neoplasms with PDGFRB or B abnormal.

Methods: Cytogenetic examination of bone marrow cells obtained from patients was performed by 24th culture method. R banding technical was used for karyotype analysis. PDGFRB and B gene rearrangement were detected by FISH using triple-color of 4q12 and dual color break-apart PDGFRB probes.

The fusion genes of rearrangements of PDGFRB and B genes were detected by RT-PCR. Immunohistochemistry analysis was carried out by flow cytometry. Most of all cases were treated with IM and followed up.

Results: The diagnoses included 27 cases of MPN, 1 case of AML-M2 and 1 case of non-hodgkin lymphoma. 21 cases were PDGFRB rearrangement, the other 8 were PDGFRB abnormal. 7 of 8 were PDGFRB fusion, one of which concurrent with DEK-CAN fused gene, and the eighth had MYO18A-PDGFRB. 7 cases of the 8 PDGFRB rearrangement had a primary abnormality with t(5;12)(q33;p13) and the other one had a secondary abnormality of AML-M2. PDGFRB and B genes rearrangement detected by FISH and multiple-RT-PCR were positive. The immunophenotypical analysis showed myeloid or lymphoid. The diagnosis achieve rapid and durable remissions with IM.

Summary/Conclusions: In summary, patients with significantly anemia and eosinophilia should be screened for the presence of PDGFRB and B rearrangements. The dual-color FISH is a simple approach and should be added into the diagnostic work-up because these patients respond to imatinib therapy, and sustained responses have been observed. The OS of PDGFRB and B abnormal was similar with a previous report in a western population and another Chinese hematology center.

**PB2042**

PLATELET AGGREGATION STUDY OF ESSENTIAL THROMBOCYTHEMIA TREATED WITH ANAGRELIDE

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Background: Essential thrombocytemia (ET) is a myeloproliferative neoplasm characterized by thrombocytosis and abnormal megakaryocyte proliferation. Patients with elevated platelet count are considered to be a high-risk group for thromboembolic and/or hemorrhagic complications. In Japan, anagrelide treatment was recently approved for the 1st line as a cell reduction therapy on ET. Even now, there are few study whether the risk of thrombosis has decreased after anagrelide treatment. Moreover, the platelet count problem uncertainty remains what is the best practice to follow when the platelet count in platelet-rich plasma (PRP) exceeds about 600x10⁹/L, in the recent recommendations of the standardization of light transmission aggregometry by the platelet physiology subcommittee of Scientific and Standardization Committee /International Society of Thrombosis and Hemostasis.

Aims: The aim of this study was to characterize the platelet aggregation (PA) in patients with ET. We would also clarify whether there were any changes of hemostatic side effect and platelet aggregability before and after treatment with anagrelide.

Methods: This study has been conducted with blood sample obtained from six healthy subjects, compared to 18 consecutive patients with ET. None of the patients was taking anticoagulants or cytoxic therapeutic agents. We also studied six healthy subjects with untreated ET. Whole blood aggregometry (WBA) and LTA using PRP were performed. ADP-induced PA or collagen-induced PA used natural count PRP and platelet count adjusted PRP with platelet-poor plasma. Data were compared in the groups using the Tukey-Kramer test. This study was approved by the Ethical committee of our hospital. All study procedures were performed in accordance with the Declaration of Helsinki.

Results: The result of WBA was not obtained, because the filter was obstructed by giant platelets. In the natural PRP, even over 900x10⁹/L, the platelet aggregability was markedly increased compared with the control (ADP-induced PA: p=0.023, collagen-induced PA: p=0.001), but, was not significantly different (ADP-induced PA: p=0.703, collagen-induced PA: p=0.986) in the count adjusted PRP. These results were not confirmed in cases with platelet counts of less than 600x10⁹/L. There was no decrease in platelet aggregation before and after treatment with anagrelide (ADP-induced PA: p=0.3403, collagen-induced PA: p=0.514).

Summary/Conclusions: In the ET patients with platelet counts more than 900x10⁹/L, the platelet aggregation by LTA with natural count PRP was remarkably accelerated and this data seemed to reflect the disease status. Although treatment with anagrelide showed cyto-reductive effect without any hemorrhagic complication in patients with ET, it did not fully reduce platelet aggregability.

**PB2043**

A SINGLE CENTRE EXPERIENCE OF MASTOCYTOSIS

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Background: Mastocytosis considered as a subcategory of myeloid neoplasms based on World Health Organization (WHO) 2016 classification, is characterized by expansion and accumulation of abnormal clonal mast cells in
one or more organs. KITD816V mutation and other KIT mutations play as driver mutations in the pathogenesis of disease. KITD816V mutation is positive in%80 of systemic mastocytosis patients across generations, will improve the understanding of this entity.

Table 1.

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 thrombocytopenia (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.

Aims: To analyze differences in treatment strategies used by physicians and 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 solely 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 treatment of 20 patients rather than delay at diagnosis of PV or ET. A greater proportion of ROSW physicians reported they would observe >25% of patients at diagnosis (UK - 54% MF, 30% PV, 37% ET; ROSW - 51% MF, 48% PV, 50% ET). No difference was observed in the most commonly received treatments for each disease, but a greater proportion of UK patients reported receiving treatments than ROSW. This difference was greatest 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 (HU) (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 rated who they thought should be the main decision maker on a scale of 1 (the physician) to 5 (the patient). ROSW physicians were significantly more inclined to be involved in treatment decisions (mean: UK, 6.25; ROSW, 7.01). UK physicians supported this as more ‘agreed strongly’ with the statement ‘I involve my MPN patient 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 were more likely 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 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 with MPN.

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 platelets (17,000/μl) 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 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 63 years). Similarly, platelet count at diagnosis tended to be higher in the subgroup of type 2 mutation carriers than in patients with the type 1 mutation while hemoglobin levels and white blood cell count were lower compared to those with non-type 2 mutation.

The mutual allele burden of JAK2V617F /CALR exon indel mutations of two PMF patients found as 10%/65% and 15%/55% respectively. In our cohort, 10% of the patients with CALR mutation had anemia, 21% had splenomegaly, and 43% had megakaryocytes at time of diagnosis. Measurement of 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 platelets (17,000/μl) 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.

Impact of CALR mutations on the clinical and laboratory features of ET and PMF has been found. Type I 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 results of this study show that the type of mutation in the Hb level and the number of WBC in ET. An important observation was the detection of the type of JAK2 V617F 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 and DIPSS.

Summary/Conclusions: The effect of the type of JAK2V617F mutation on the clinical and laboratory features of the ET and PMF has been found. Type I 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 type of JAK2 V617F 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.

PB2047
IMPACT OF THE TYPE OF CALR MUTATIONS ON THE CLINICAL AND LABORATORY FEATURES OF ESSENTIAL THROMBOCYTHEMIA AND PRIMARY MYELOFIBROSIS
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Background: In 2013, in the majority of JAK2V617F negative patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) have been identified mutations in the 9 exon of CALR gene. Described more than 30 different mutations, subdivided into two subtypes: deletions (type I) and insertions (type II). There are data on the phenotypic effects, depending on the version of CALR mutations. However, the prognostic significance of mutations CALR is still insufficiently clear.

Aims: To assess the impact of the type I and type II mutations of CALR on the clinical and laboratory features of ET and PM.
Background: Patients with myeloproliferative neoplasms (MPNs), including myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated patient-reported impact of MPNs across 6 countries. Aims: To analyze differences in disease and symptom burden of MPN patients between the UK and the Rest of the 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 (UK, n=103; ROW, n=116) 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 5.74), pain (mean: UK 8.09, ROW 5.83), and loss of concentration (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 (37% in the UK compared to 16% in the ROW). UK patients had an average overall symptom burden score of 40.1 compared with 24.1 among ROW patients. UK patients were also more likely to have been classified with a high risk score at diagnosis (UK 22% vs ROW 9%). Despite the consistently greater symptom burden experienced by UK patients, little difference was observed in patient satisfaction with their healthcare and level of communication (UK 81% satisfied vs 90% disease management (UK 87%, ROW 90%). However, UK patients were more likely to disagree with the statement ‘My doctor understands how much my condition impacts my life’ (UK 39% vs 22% ROW). UK physicians had more MPN patients under their care than ROW (mean patients under care in last 12 months: UK 25, ROW 15). 25% of MPN patients in ROW were not currently receiving treatment compared to 12% in the UK.

Summary/Conclusions: Data presented here will inform next steps for a RCT investigating the effectiveness of online yoga for symptom management in MPN patients.

PB2050

COMPARISONS OF SYMPTOM BURDEN IN MYELOPROLIFERATIVE NEOPLASMS 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 thrombocythemia (ET), experience a substantial disease burden. The international MPN LANDMARK survey evaluated patient-reported impact of MPNs across 6 countries.

Aims: To analyze differences in disease and symptom burden of MPN patients between the UK and the Rest of Surveyed World (ROWS).

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 (UK, n=103; ROW, n=116) 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 5.74), pain (mean: UK 8.09, ROW 5.83), and loss of concentration (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 (37% in the UK compared to 16% in the ROW). UK patients had an average overall symptom burden score of 40.1 compared with 24.1 among ROW patients. UK patients were also more likely to have been classified with a high risk score at diagnosis (UK 22% vs ROW 9%). Despite the consistently greater symptom burden experienced by UK patients, little difference was observed in patient satisfaction with their healthcare and level of communication (UK 81% satisfied vs 90% disease management (UK 87%, ROW 90%). However, UK patients were more likely to disagree with the statement ‘My doctor understands how much my condition impacts my life’ (UK 39% vs 22% ROW). UK physicians had more MPN patients under their care than ROW (mean patients under care in last 12 months: UK 25, ROW 15). 25% of MPN patients in ROW were not currently receiving treatment compared to 12% in the UK.
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 feel they don’t have enough time to discuss all symptoms. This is likely to be impacting on the ability of patients and physicians to communicate fully on symptoms and to agree on the best disease management plan.

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: MPN Symptoms Assessment Form Total Symptom Score (MPN-SAF TSS) was assessed as mean/median of 10 items: fatigue, concentration, early satiety, inactivity, night sweats, itchig, bone pains, abdominal discomfort, weight loss and fever. JAK2V617F and exon12 mutations were performed by allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) while CALR and MPL W515mutations were assessed by high-resolution melting (HRM).

Results: 93 MPN patients (48 males and 45 females): 18 polycythemia vera (PV), 41 essential thrombocythemia (ET), 24 primary myelofibrosis (PMF), 10 Post-ET/PV-myelofibrosis (post-ET/PV-MF) were included. Median age at diagnosis was 55 years (17-75) and was lower in ET than PV and PMF patients; 44 (19-75) vs 56 (34-70) years and 56 (20-75) years, respectively (p<0.001). JAK2 mutation was positive 53/93 (57%); 16 (90%) PV patients, 14 (34%) ET patients, 15 (62%) PMF patients, 8 (80%) post-ET/PV-MF patients (p<0.001). CALR mutation was positive in 14/93 (15%); 10 (24%) ET patients, 4 (17%) PMF patients, zero (0%) Post-ET/PV-MF patients (p=0.050). MPL mutation was positive in 3/93 (3%); 2 (5%) ET patients, 1 (4%) PMF patients, zero (0%) Post-ET/PV-MF patients. 23/93 (25%) patients were triple negative; 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-ET/PV-MF (p<0.001). From 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 36 (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). OS 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 MPN patients. Triple negative ET patients in our cohort have significantly lower MPN10 score, show lower incidence of BM fibrosis and splenomegaly which may indicate a more indolent disease course.

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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Background: Interferon-alpha (IFNa) based therapies have been successfully used in myeloproliferative neoplasms for over thirty years. A known burden for long-term therapy applying IFNa in otherwise fit outpatient is the necessity of frequent hospital visits for product administration. Ropeginterferon alfa-2b (AOP2014) is a novel long-acting monopegylated IFNa allowing initially bi-weekly and, in long-term maintenance, monthly administration. To further improve on convenience and compliance, a pre-filled, dose-adjustable pen was developed for patient self-administration at home.


Methods: The study was performed in 18 sites in 8 European countries. Patients were eligible who completed the AOP2014-arm in the PROUD-PV study (12 months of treatment). A total of 7 visits was scheduled within 3 months (before the supervised self-administration at site, followed by 6 supervised visit at 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% (28/36) 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 the full 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 second supervised visit at site and beyond. 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. For the AOP2014 pen itself (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) most of the patients judged the product “good” or “good enough”. 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 AEs were moderate to mild severity. One serious AE (anemia, unrelated), one pen-related 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.
Summary/Conclusions: The AOP2014 pen was well accepted and no major difficulties were reported. The study drug performed as expected and there were no safety concerns arising from the administration of AOP2014 using the pen device. The AOP2014 pen allows for individual dosing and a patient-convenient mode of self-administration of ropeginterferon alfa-2b at home and is expected to support adherence and compliance in the long-term treatment of PV patients.

JAK2, CALR AND MPL MUTATIONS: CORRELATION WITH PHENOTYPE DISEASE AND HISTOPATHOLOGICAL FEATURES OF BONE BIOPSY

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Background: Drivers mutations JAK2, CALR and MPL are mutually exclusive in Essentials thrombocytemia (ET) and these are included in the diagnostic criteria of mieloproliferative neoplasms (MPNs). Consistent with known literature, the molecular characterisation have implications in the phenotipo 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 megacycocytes according with the mutational status and there were more frequently in patients with CALR mutantation (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.

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<tr>
<th>No.</th>
<th>JAK2</th>
<th>CALR</th>
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<th>Follow-up JAK2</th>
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Summary/Conclusions: In our study we can confirm that there are differences between clinical and laboratory finding according with mutational status, as shown in previous studies. The most consistent finding of this study was the presence of laxes groups of megacycocytes significantly higher in those with CALR mutations. The major limitations of this study include a small number of patients and biopsies available to analysed, this might be the mayor causes for the lack of the data demonstrating clinical and histological relevance. But our results should not be underestimated because, to our knowledge, this is the second study thus has investigated this relation.

**PB2055**

**CLINICAL IMPLICATION OF QUANTITATIVE JAK2 V617F ANALYSIS WITH DROPLET DIGITAL PCR IN MYELOPROLIFERATIVE NEOPLASMS**

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Background: JAK2 V617F is the most common genetic mutation in myeloproliferative neoplasms (MPN) and included in the major diagnostic criteria. Beyond the description of existence, quantification of mutational load is proposed as a useful information to classify subgroups of MPN and to predict prognosis. Droplet digital PCR (ddPCR) is a novel assay which has an advantage in accurate and reproducible quantitative analysis.

Aims: This study was planned to verify the correlation of ddPCR with pyrosequencing in diagnosis of MPN and to investigate clinical implication of the mutation burden in disease course.

Methods: Between 2012 and 2016, peripheral blood or bone marrow samples were obtained from 56 patients at diagnosis and every 3 months after enrollment. Inclusion criteria were 1) older than 20 years, 2) who were newly diagnosed with MPN and 3) diagnosed with MPN before, not met the indication of JAK2 inhibitor treatment yet. JAK2 V617F mutation was detected by pyrosequencing as diagnostic work-up. The ddPCR was performed using the same samples with pyrosequencing to prove correlations between assays and to establish a detection sensitivity cutoff. Clinical aspects and hematologic profiles of enrolled patients were reviewed.

Results: The lowest value of measured JAK2 V617F allele by ddPCR except negative samples in our study was 0.01%, which was approximately 0.07 copies/μL of mutant allele. Some discrepancies were observed from 0.0001% to 0.01% concentration between the expected and measured values in ddPCR detection sensitivity assay. 0.1% was determined as the cutoff. Forty-two patients (75%) were positive for JAK2 V617F by pyrosequencing and 46 (82.1%) were positive by ddPCR. The median mutant allele at diagnosis was 37.5%±30.08%. With ddPCR, the mean was 40.7%±31.2%. Pyrosequencing and ddPCR were highly correlated (r=0.9712, P<0.001). JAK2 V617F burden measured with ddPCR was significantly different by subgroups (P=0.001). In comparison of one disorder with another, polycythemia vera (PV) had more amount of mutant allele than essential thrombocytosis (ET) (P=0.001), however, differences between PV-myelofibrosis (MF) and ET-MF were not statistically significant. Follow-up samples were available in 12 patients and 8 were JAK2 V617F positive. Among them, reduction of mutant burden after treatment was observed in 6 patients (75%). JAK2 V617F burden showed initial reduction in a MF patient treated with JAK2 inhibitor, however, after dose reduction for toxicities, the JAK2 V617F mutation increment with hematologic aggravation was discovered. Mutation burden decrease showed a tendency consistent with hematologic improvement. Hematologic characteristics and JAK2 V617F load at the initial diagnosis and follow-up after treatment (Table 1, Figure 1).

Table 1.

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* Data from the next follow-up sample. † Data from the first follow-up sample.

Figure 1.

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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 cells in their peripheral blood. These patients fulfill the criteria 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 best treatment and to find a potential protective role of CALR mutation given the absence of thrombosis in IPSS and IPSET categories.

Methods: We collect 317 patient’s blood lab results in 433 DS patients. 102 patients have 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. Only one patient progressed to Acute Myeloid Leukemia (AML) in 98 days of his life. Other 6 patients have blast in their blood after three months of life, and underwent chemotherapy due to hematologic malignancy. All patients with leukemia has anemia at diagnosis, which is not found in TMD patients (p=0.018). In 7 leukemia patients, 3 was acute lymphoblastic leukemia (ALL) and 4 acute myeloid leukemia. All AML patients had AML1-ETO rearrangement, additional to trisomy 21 at their diagnostic point, which did not 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.

PB2058

INFECTIONOUS EVENTS IN A COHORT OF PATIENTS WITH MYELOFIBROSIS UNDER TREATMENT COMPARING RUXOLITINIB WITH CONVENTIONAL THERAPY, A MONOCENTRIC EXPERIENCE OF 22 PATIENTS RETROSPECTIVELY ANALYZED

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1Department of Hematology and Bone Marrow Unit, Pescara, 2Section of Hematopathology, Department of Hematology and Oncological Sciences, S. Orosia-Malpighi Hospital, Bologna, Italy

Background: Previous studies have shown that ruxolitinib therapy was associated with increased risk of infection, but the incidence and clinical relevance of these infections among patients with myelofibrosis (MF) treated with ruxolitinib are not well described.

Aims: The aim of this study was to retrospectively analyze the incidence and clinical relevance of infectious events among patients with MF treated with ruxolitinib in a single center.

Methods: The study population consisted of 22 MF patients treated with ruxolitinib at our center from January 2012 to December 2016.

Results: A total of 340 infections were reported in 22 patients during the study period. The most common infections were respiratory tract infections (30.9%), followed by skin and soft tissue infections (21.7%). The incidence of serious infections (SIs) was 13.6% during the study period, with the majority occurring in the first year of treatment. The incidence of hospitalizations due to infections was 59.1%, with 44.1% of patients requiring ICU admission.

Summary/Conclusions: In patients with MF treated with ruxolitinib, the incidence of infections and SIs was significantly higher compared to historical controls. These data highlight the need for close monitoring and prophylactic measures to mitigate the risk of infections in this population.
Background: Treatment with the Janus-activated kinase (JAK) 1 and 2 inhibitor ruxolitinib decreases constitutional symptoms and spleen size in myelofibrosis. However accumulating evidences suggest that the drug also exerts substantial immunosuppressive activity. The impressive clinical activity of ruxolitinib is predominantly mediated by its profound anti-inflammatory effects modulating dendritic cell (DC) function resulting in impaired CD4+ and CD8+ activity. Several studies have shown that Ruxolitinib affects different cytokines (IL1, IL6 and TNFα/α) 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 were low risk, 10 were intermediate risk and 4 were high. A total of 5 documented infections were identified throughout the evaluation period, 4 were grade 1 and one grade 2. They are various including oral herpes simplex reactivation, pneumonia, recurrent viral flu syndromes, esophagitis fungal and urinary infections. All of them were present in the subgroup of patients undergoing therapy with Ruxolitinib (45%) after a medium time of 8 months from beginning of therapy (range 3-10). No patients received any anti-infective prophylaxis. Median total daily dose of ruxolitinib was 10 mg (range 5-20). All of this infections were resolved after antimicrobial therapy and did not require hospitalization. None of patients were treated with concomitant immunosuppressive therapy. 3 of this patients presented renal impairment (median creatinine clearance of 46 ml/min).

Summary/Conclusions: These data in our small series of patients suggest a higher incidence of ruxolitinib associated infections observed in clinical practice compared to traditional treatment. Immunosuppressive effect of Ruxolitinib is reported and the use of this drug in the transplant setting with beneficial effects on alloreactivity and on graft versus host disease is becoming more common. These patients might benefit from receiving prophylactic therapy with antiviral drugs or antibiotics or antifungal therapy or in alternative by careful monitoring. Finally nowadays physicians and patients should be aware of potential risks of using ruxolitinib including the risk of infections.

In summary, infections can occur in patients treated with ruxolitinib but are generally mild. Generally infections were non-life threatening and managed with appropriate supportive care. Special care probably should be taken for patients older (more than 75 years old), treated with corticosteroid and with renal impairment. However larger studies are needed to confirm these observations.
platelet disorders, acquired deficiency of factors V and VWF, disseminated intravascular coagulopathy, thrombocytopenia, hemorrhagic diathesis.

**Aims:** The aim of this study is to monitor the count of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values as potential risk factors for the incidence of hemorrhagic complications in patients with chronic myeloproliferative neoplasms.

**Methods:** During the three-year period we monitored the occurrence of hemorrhagic complications in 139 patients of both sexes, aged between 30 and 87 years, being diagnosed with Ph-myeloproliferative neoplasm. Patients were classified into the following groups: 1. Group with polycythemia vera (PV) (61); 2. Group with essential thrombocythemia (ET) (28); 3. Group with idiopathic myelofibrosis (IMF) (25); 4. Group with unclassified myeloproliferative neoplasms (MPNs) (25). The following possible risk factors were monitored: counts of erythrocytes, leukocytes and platelets, as well as hemoglobin and hematocrit values. 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<0,001) and the lowest in the group of patients with IMF (p>0,01). Among the groups of patients with PV and MPNs there was no statistically significant difference in those parameters. In the group of patients with PV and MPNs hemorrhagic complications were more frequent in percentage in patients with leukocytosis and erythrocytosis, but without statistical significance. The highest platelet count was found in the group of patients with ET and MPNs (p<0,001), and the lowest in the group of patients with IMF (p>0,01). Among the group of patients with PV and MPNs there was no statistically significant difference with regard to platelet count. Hemorrhagic complications were more frequent both in patients with platelet count below 10x10^9/L (p<0,05) and in patients with platelet count over 1000x10^9/L (p<0,01). The increase in platelet count influences the adsorption of larger von Willebrand multimers on the platelet membrane, thus having an effect on their elimination from circulation and degradation.

**Summary/Conclusions:** The platelet count can be considered a significant parameter for monitoring the risk of hemorrhagic complications in patients with myeloproliferative neoplasms, particularly with ET and IMF. Deviation from the count of leukocytes, erythrocytes, hemoglobin and hematocrit values may be considered as a potential risk factor for bleeding in patients with myeloproliferative neoplasms, but further follow-up and a larger number of subjects are needed. The age of the patient can also be considered as a risk factor for the incidence of hemorrhagic syndrome in those patients. The follow-up of patients with unclassified myeloproliferative neoplasms has been particularly important, which showed a high prevalence of hemorrhagic complications, and with the purpose of their further differentiation.

PB2062

**CLINICAL RELEVANCE OF JAK2V617F MUTATIONAL LOAD IN PATIENTS WITH PHILADELPHIA NEGATIVE MYELOPROLIFERATIVE NEOPLASMS FROM REPUBLIC OF MACEDONIA (SINGLE-CENTER EXPERIENCE)**

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1Hematology, University Clinic of Hematology, Skopje, Macedonia, The Former Yugoslav Republic Of

**Background:** Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are Philadelphia chromosome negative myeloproliferative neoplasms (MPN) characterized by the expression of an activated JAK2V617F mutation. Up to date, it remains controversial how one mutation can lead to expression of three different clinical MPN phenotypes. However, several studies have shown that the Jak2V617F allele burden may correlate with specific MPN entity.

**Aims:** In order to further clarify these observations, we evaluated the Jak2V617F mutational status and its clinical implications in 233 Jak2V617F+ patients with different MPNs from the Republic of Macedonia.

**Methods:** We conducted a single center retrospective study which included 233 patients with Jak2V617F+MPN diagnosed according to WHO criteria, with molecular follow up of 4 years. Quantification of the Jak2V617F mutation was analyzed with the Real Time PCR method using the Larsen protocol. Based on the mutational load patients were divided in three groups: first with <10% mutational load, second with 10-50% load and third with >50% mutational load. The correlation of the allele burden with various clinical parameters was done by univariate's tests using Statgraphics 4.3 software.

**Results:** Our study showed that median allele burden was lowest in patients with ET (22.8%), followed by PV patients (37.1%) and PMF pts (49.6%) (p<0,01). A higher mutation burden (>50% vs <10%) was associated with advanced age (67.5 vs 58.5 years and 65 vs 58 years in ET and PMF pts respectively), with higher leukocyte count (10.23x10^12 vs 8.87 13.8 vs 12.4, and 5.8x10^12 in ET, PV and PMF pts respectively), with elevated erythrocyte count (5.76 vs 4.85 and 5.59 vs 4.52 in ET and PMF pts respectively), and with higher hemoglobin level (g/dL) and platelet count 10^12/L (15.45 vs 14.35 and 1071.5 vs 860.5 in ET patients respectively) (p<0,05 for all comparisons)

**Conclusion:** Our study confirmed that higher allele burden is associated with advanced age, polycythemic features, and increased leukocytes and platelets counts regardless of the diagnosed MPN entity and suggests that implementation of JAK2V617F allele burden in the diagnostic workup of MPN pts could help for choosing the optimal treatment option in this group of patients.

PB2063

**CSF3R T618-MUTATED CHRONIC NEUTROPHILIC LEUKEMIA: A RARE CASE SUCCESSFULLY TREATED WITH RUXOLITINIB**

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1Division of Hematology, A.O. “Policlinico-Vittorio Emanuele”, University of Catania, Via Citelli 6, 95124 Catania, Italy, Catania, Italy

**Background:** Chronic neutrophilic leukemia (CNL) is a rare BCR-ABL1- negative myeloproliferative neoplasm (MPN) with only 200 patients reported to date according to the WHO criteria. These cases are characterized by a high number of mature neutrophils in peripheral blood (PB), a hypercellular bone marrow due to neutrophilic granulocyte proliferation and hepatosplenomegaly. None standard of care exist for CNL: most patients are palliated with hydroxyurea, interferons, splenic radiation or splenectomy.

In the last years CNL has been considered a chronic myeloid leukemia (CML), atypical CML (aCML) or chronic myelomonocytic leukemia (CMMI), however, this diagnosis has been more defined since the oncogenic mutations in the granulocyte colony-stimulating factor receptor (CSF3R) gene were identified in approximately 83% of WHO-defined CNL patients. CSF3R T618I mutation is now considered as a highly specific molecular marker for CNL that is sensitive in vitro and in vivo inhibition by currently approved protein kinase inhibitors.

**Aims:** Here we report a case of a 76-years old man with diagnosis of chronic neutrophilic leukemia, according to WHO criteria, successfully treated with ruxolitinib.

**Methods:** On May 2015 a 76 aged male patient presented at our Institution with fatigue, night sweats, neutrophilic leukocytosis (neutrophils 42.080/mm, immature granulocytes <5%), and symptomatic splenomegaly (277x127x200 mm). The initial leukocyte count was 27x10^9/L and the hemoglobin level was 9,9 gr/dl) and thrombocytopenia (82.000/mmc); meanwhile polymerase chain reaction (PCR) studies revealed the presence of CSF3R T618I mutation, suggesting diagnosis of CNL. By taking into account the activity of ruxolitinib in overt PMF, we decided to start this drug. The initial dose was 5 mg twice daily on a follow-up of 6 months after initiation of ruxolitinib therapy, symptoms resolved, hemoglobin and platelet levels improved (PLT 186.000/mmc), leukocytosis persisted (WBC 24.600/mmc), and the patient achieved a dramatic reduction in spleen size (209x119x74 mm).

**Summary/Conclusions:** Current data suggest that constitutively active JAK-STAT signaling plays a central role in the pathogenesis of BCR-ABL1-negative myeloproliferative neoplasms (MPNs); our experience suggests that ruxolitinib use in CNL patients can induce partial responses by improving marrow function (normalization of hemoglobin and platelet counts), splenomegaly and symptoms.

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PERIPHERAL BLOOD CELL STUDY FROM PATIENTS WITH FOLLICULAR LYMPHOMA AND DIFFUSE LARGE B-CELL LYMPHOMA: WHAT SHOULD WE EXPECT?

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1Haematology, Hospital Universitario de Guadalajara, Guadalajara, 2Haematology, Hospital Universitario Puerta de Hierro, Madrid, Spain

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).

Background:

PB2065

THE ACQUISITION OF RESISTANCE TO BENDAMUSTINE HYDROCHLORIDE INDUCES MULTIDRUG RESISTANCE IN A NOVEL MANTLE CELL LYMPHOMA-DERIVED CELL LINE KUMP-YPU1

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Background: Bendamustine hydrochloride (BH) has been one of the most promising chemotherapeutic agents for mantle cell lymphoma (MCL), however, its mechanisms of action and the mechanisms for the acquisition of resistance to BH have not been fully clarified.

Aims: We tried to identify the underlying mechanisms for BH resistance to develop the strategy to overcome BH resistance.

Methods: This study was conducted in accordance with the Declaration of Helsinki and with the approval of the Institutional Review Board. Patient’s sample was obtained along with the written informed consent. We firstly established a novel MCL-derived cell line, KUMP-YPU1, from circulating lymphoma cells of a 77-year-old male patient with MCL. A BH-resistant subline of KUMP-YPU1 was established by continuous exposure to BH with gradual escalation of its concentration from 5 μM up to 50 μM for about 8 months. Cyto- genetic analysis was performed by double color-fluorescence in situ hybridization and spectral karyotyping (SKY). The comparative gene expression profile (GEP) and the ingenuity canonical signal pathway analyses between of KUMP-YPU1 and KUMP-YPU1R were performed to identify the differential gene expression pattern along with the acquisition of BH resistance. Cell viability was evaluated by a modified MTT assay.

Results: SKY analysis revealed that both primary tumor cells and KUMP-YPU1 had complex karyotype involving three-way translocation (8;14;11) (q24;q32;q13), involving the rearrangement of MCL1, CD23 and MAF, respectively. By GEP analyses, total of 472 genes were differentially expressed in KUMP-YPU1R compared with KUMP-YPU1. Among these, 241 genes were upregulated more than 1.5-folds and 160 downregulated less than 0.67-folds in KUMP-YPU1R. The deregulated activation of a Ser/Thr kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1) has been shown to promote the disease progression in various solid cancers. In hematologic malignancies, we have recently identified that the constitutive activation of PDK1 and its downstream kinase RSK2 strongly correlates with multiple myeloma (MM) pathophysiology by promoting myeloma cell survival and proliferation (Chinen Y, Cancer Res 2014; Shimura Y, Mol Cancer Ther 2012). Mantle cell lymphoma (MCL) is cytokinetically 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 cells.

In this study, we examined 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 biopsy specimens were obtained with informed consent and subjected to the immunohistochemical (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(Ser241), p-RSK2(Ser380), and RSK2. BX-912, a specific inhibitor for PDK1, was purchased from Selleckchem (USA). RNA interference of PDK1 was performed by transfection of short hairpin RNA plasmids into MCL cells 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 noted 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 4 MCL cell lines (IC50 0.9–2.5 mM), and this inhibitory effect of BX-912 was more profound in MCL cell lines compared with three DLBCL cell lines (IC50 3.7–17.0 mM) and a BL cell line (IC50 2.9 mM). In addition, the flow cytometric analysis revealed that the growth inhibition of MCL cells by PDK1 knockdown was BX-912 resistant but was not observed in a BL cell line. This suggests that PDK1 knockdown may be a promising therapeutic approach for MCL.

Summary/Conclusions: Collectively, our study suggested that PDK1/RSK2 signaling axis is the potential therapeutic target in MCL.

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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).

Results: No difference in the distribution by sex or age was found between patients with FL and DLBCL. A low cell count in at least, one lymphocyte population was detected in 35/52 patients (67.3%). 100% of cases had a low number of polyclonal B cells (<100/ml). Comparison of low-grade FL, grade 3 FL and DLBCL did not show any statistically significant difference regarding monocytic cells, CD4 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 CD3 + CD8 + T cell population (1.5±4.29 versus 2.06±1.44, p=0.002), and circulating mononuclear B cells, for both percentage (15.2±23.25 versus 1.94±23, p=0.001) and absolute number (869±1758 versus 18.75±56.47, p<0.001). Grade 3 FL and DLBCL also showed a different CD4:CD8 ratio (1.16±0.45 versus 2.06±1.44, p=0.001), with a trend toward significance regarding CD4 T cells (413±18 versus 685±457, p=0.077). Grade 3 FL showed a lower number of polyclonal B cells as compared to DLBCLs (664±41 versus 105±102, p=0.048). The cell proliferation in the expression of monocytic B cells was higher in low-grade FL than in grade 3 FL, in both percentage (15.2±22.3 versus 4.58±28.8, p=0.008) and number (862±1758 versus 43.36±69.91, p=0.002) of monoclonal B cells. The number of lymphocyte subpopulations versus with low-cell counts was higher in grade 3 FL than in low grade FL (p=0.03).

Summary/Conclusions:

The peripheral lymphocyte profile in patients with FL and DLBCL is heterogeneous, but B-lymphopenia and CD4:CD8 ratio deviations are frequent findings. Regardless of clinical stage, low-grade FL had more circulating lymphoma cells and preserved lymphocyte populations than grade 3 FL. Further studies are warranted to confirm these exploratory findings and determine their clinical implications.
Aims: This study suggests that the detection of atypical lymphocytes only in BM aspiration had no significant difference compared with BM involvement in the development of BH resistance in MCL. In addition, BH-resistance cells were also found to express decreased mRNA level of ASS1 and MGST1 in the development of BH resistance in MCL cells. Atypical lymphocytes only in BM aspiration and those with no 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 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.

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. Therefore, the development of strategy which overcomes the treatment refractoriness in MCL.

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 involvement 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.

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 nuclear vacuolization and aggregation including lymphoid aggregates or 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 in 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 in both BM aspiration and biopsy. 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.

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 nuclear vacuolization and aggregation including lymphoid aggregates or 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.

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PB2069
THE PROGNOSTIC ROLE OF INDOLEAMINE 2,3-DIOXGENASE EXPRESSION IN HODGKIN’S LYMPHOMA.

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Background: Indoleamine 2,3-dioxygenase (IDO) is an inducible enzyme that catalyzes the initial and rate-limiting step in tryptophan along the kynurenine pathway. IDO is a key factor maintaining immune tolerance and expression and it correlates with poor clinical outcome in different types of cancer and hematological malignancies. It also plays a role in a lot of pathophysiological processes, such as antitumor and antimicrobial defense. IDO causes immunosuppression in the tumor microenvironment by tryptophan breakdown. Although, only several reviews have been made to evaluate IDO expression value and its expression value in hematological malignancies.

Aims: The aim of the study was to assess the impact of the IDO expression on clinical course of lymphoma, with 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 of disease and IDO expression in our study. Patients with the absence of IDO expression tended to have a better response to the 1st line chemotherapy comparing to patients with positive IDO expression. The overall response rate was achieved in 71.4% (5/7) of IDO+ cases and in 92.9% (26/28) of IDO- cases. The relapse rate was more frequently found in HL cases with IDO+ compared to IDO- cases (28.5% (2/7) versus 7.1% (2/28), respectively, p=0.05). We did not register any death of patients in IDO+ group, while one patient in IDO+ group died during the follow-up period (median duration – 37 months; range 10-65 months). The presence of IDO expression in pre-treated HL biopsies was associated with the reduced event-free survival (EFS) in HL patients. A 4-year EFS rate for IDO+ HL patients was 50% compared with 73% for IDO-negative HL patients (p=0.002). The prognostic significance of IDO* expression in clinical outcome of HL (EFS) was also confirmed by multivariate analysis (HR=2.9; 95%CI 0.8-10.1; p=0.006).

Summary/Conclusions: On the base of the study, our findings suggest that IDO might be a promising marker for HL prognosis as well as represents an attractive target for HL immunotherapy in patients with poor outcome.

PB2071
IGHV SOMATIC MUTATION PROFILE AS PATHOGENETIC SIGNATURE IN SPLENIC MARGINAL ZONE LYMPHOMA AND SPLENIC DIFFUSE RED PULP LYMPHOMA

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Background: Splenomegaly (SMs) are rare chronic lymphoproliferative neoplasms with a very indolent clinical course and a non-characteristic phenotype and karyotype. According to the World Health Organization (WHO) the most common type of SMs is splenic marginal zone lymphoma and characterized by a peculiar morphology with micronodular pattern of infiltration, biphasic cytology, and the almost constant presence of marginal zonal differentiation. Splenic diffuse red pulp lymphoma been introduced as a provisional entity but differential diagnosis with other SMs is needed to be performed. In the therapeutic strategy the knowledge on the clonal evolution of the disease is very important.

Aims: The aim of our study to determine the immunoglobulin variable heavy chain (IGHV) gene usage and somatic mutation patterns in a series of SMZL and SDRP patients.

Methods: We studied 24 patients with SMZL, 40 patients with HCL and 10 patients with SDRP. Diagnosis was based on standard WHO classification criteria. In all cases, the diagnosis was based on peripheral blood and BM findings. The baseline clinical and laboratory features as well as follow-up and outcome were recorded for each patient. Rearranged IGHV genes were amplified essentially in reactions that contained only one of the 5’ leader region primers for the indicated VH 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 revealed that most cases use the VH1 gene family. In SDRP all cases were different from the VH1 family. 16 the VH1 family segments. The VH1 family segments were used in 16 cases. In 4 out of 24 cases (16.67%), IGHV genes were in germline or near germline configuration, whereas in 20 cases (83.33%), IGHV genes were somatically mutated. We have no shown differences in clinical and laboratory characteristics, immunophenotype, outcome or overall survival were observed between the mutated and unmutated cases of SMZL. A comparison of the VH genes to reported germline sequences in SDRP revealed that five cases used the VH3 family VH gene segments and five the VH4 family, one of case with unmutated IGHV genes. Summary/Conclusions: Our analysis also showed the selective use of VH1 family in SMZL cases. 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 of disease and IDO expression in our study. Patients with the absence of IDO expression tended to have a better response to the 1st line chemotherapy comparing to patients with positive IDO expression. The overall response rate was achieved in 71.4% (5/7) of IDO+ cases and in 92.9% (26/28) of IDO- cases. The relapse rate was more frequently found in HL cases with IDO+ compared to IDO- cases (28.5% (2/7) versus 7.1% (2/28), respectively, p=0.05). We did not register any death of patients in IDO+ group, while one patient in IDO+ group died during the follow-up period (median duration – 37 months; range 10-65 months). The presence of IDO expression in pre-treated HL biopsies was associated with the reduced event-free survival (EFS) in HL patients. A 4-year EFS rate for IDO+ HL patients was 50% compared with 73% for IDO-negative HL patients (p=0.002). The prognostic significance of IDO* expression in clinical outcome of HL (EFS) was also confirmed by multivariate analysis (HR=2.9; 95%CI 0.8-10.1; p=0.006).

Summary/Conclusions: On the base of the study, our findings suggest that IDO might be a promising marker for HL prognosis as well as represents an attractive target for HL immunotherapy in patients with poor outcome.
CELL OF ORIGIN ASSIGNMENT USING IMMUNOHISTOCHEMISTRY IS INFLUENCED BY BCL-2 EXPRESSION IN DLBCL PATIENTS TREATED WITH CHEMOTHERAPY

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Background: Diffuse Large B-cell Lymphoma (DLBCL) is a heterogenous disease with variable clinical and pathologic presentations. Using gene expression profiling or Lymph2Cx assay, DLBCL can be assigned as germinal center (GCB) or non-germinal center (Non-GCB) subtype. However such assays remain cumbersome or unavailable for routine clinical care. Immunohistochemical (IHC) algorithms, such as the one proposed by Hans et al., are easy to use but demonstrated variable concordance to gene expression profiling. Importantly, cell of origin (COO) assignment appears to influence overall survival (OS) but not progression free survival (PFS). Furthermore, antiapoptotic BCL-2 oncogene expression confers prognostic significance in GCB DLBCL but its significance in Non-GCB is unknown.

Aims: To examine the prognostic impact of cell of origin (COO) assignment in conjunction with BCL-2 expression in a cohort of DLBCL patients 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 rank. Relapse, progression or death was considered an event for PFS estimation. Analysis was computed using JMP software, version 11.

Results: A total of 122 patients were identified and analyzed. Median follow up of the cohort was 21.8 (1.47 – 107) months, during which OS was 73.5% and PFS was 59.9%. Stratified by COO, 2-year OS was 85%, 76.3%, 72% and 49.5% for low, low-intermediate, high-intermediate and high risk patients, respectively (p=0.006). After stratifying patients to GCB and Non-GCB, baseline characteristics between the strata with regards to gender, age, stage, extranodal disease, lactate dehydrogenase (LDH), International Prognostic Index (IPI) and BCL-2 expression were not significantly different. At 2-years, PFS was significantly higher for GCB vs Non-GCB at 72.5% vs 56.2%, respectively (p=0.002). Interestingly, BCL-2 expression predicted OS irrespective of COO assignment. Patients with BCL-2 expression had a 2-year OS of 55.6% vs 56.2% for GCB and non-GCB, respectively. Whereas, patients without BCL-2 expression has a superior 2-year OS at 79.9% vs 78.3% for GCB and non-GCB, respectively (p=0.02).

Summary/Conclusions: COO assignment using IHC demonstrated superior PFS for GCB over non-GCB however this was mitigated by BCL-2 expression. This raises questions regarding the currently presumed pathogenesis of the different subtypes and how to utilize the currently available targeted therapies including BCL-2 inhibitors. These observations warrant further study.
real-time polymerase chain reaction (qRT-PCR) was used to confirm the results of six upregulated and two downregulated lncRNAs. Bioinformatic analysis (gene ontology analysis, pathway analysis and network analysis) was performed to predict the biological functions and potential mechanisms of the differentially expressed lncRNAs in GCB DLBCL.

**Results:** We demonstrated that 21,539 lncRNAs were expressed in all samples analyzed, of which 1,548 lncRNAs were upregulated and 2,671 lncRNAs 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, a lncRNA-mRNA co-expression network was constructed to identify potential target genes related to the 3 upregulated and 2 downregulated lncRNAs.

**Summary/Conclusions:** Our data suggested that lncRNAs may play an important role in the pathogenesis of GCB DLBCL, and profile of lncRNAs may be used as a potential biomarker in the diagnosis of DLBCL and predicting its clinical outcome.

**PB2075**

**FLOW CYTOMETRY IN EVALUATION OF EXTRANODAL LYMPHOMA PRESENTING AT UNUSUAL LOCATIONS COMPARED TO NODAL LYMPHOMAS**

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**Background:** Immunophenotyping is a fundamental step in the diagnosis of hematologic lymphomas arising at extranodal sites. Flow cytometry is the gold standard in this setting due to its sensitivity, specificity, and ease of sample handling. In recent years flow cytometry (FCM) has proven useful in the evaluation of nodal and extranodal lymphoproliferative disorders on samples obtained by surgical specimens or fine needle aspiration cytology (FNAC). For years FNAC has been used for initial evaluation of suspected hematolymphoid neoplasms. Flow cytometry can additionally help in identifying B or T cell nature of neoplastic cells, clonality in case of B-cell neoplasms and any aberrant phenotype. The possibility of detecting CD20 status can help in initiating targeted therapy without undergoing tissue biopsy to do so. FNA cytology with Flow cytometry can serve as a replacement for open biopsy and may help in eliminating the need for more invasive procedures. In this study FCM analysis on cytological specimens, including nodal and extranodal mass from GIT, Thyroid, Kidney, Breast, Tonsil, cerebrospinal fluid and ascitic fluid, was performed.

**Aims:** The aim of our study was to evaluate the efficacy of flow cytometer for the evaluation of extranodal and nodal lymphomas on 40 patients with a clinical suspicion of hematolymphoid neoplasms. Samples for flowcytometric immunophenotyping (FCI) were obtained by fine needle aspiration (FNA) or by tissue scraping along with samples for cytomorphological, histological and immunohistochemical (IHC) evaluation. Samples collected in saline were submitted for FCM on 5-color Beckman Coulter FC-500, using a set of mature and immature antigens markers for lymphoid cells. Results of FCM on cytological specimens along with cytological findings were compared with histological and IHC diagnosis.

**Results:** Flowcytometric immunophenotyping conducted on extranodal sites included total 10/40 (25%) cases out of which most common site was GIT (4 cases) followed by CNS (3 cases), Kidney (1 case), Thyroid (1 case), Breast (1 case), and Tonsil (1 case).Definite diagnosis using only FCI could be obtained in 25/40 (62.5%) cases in which 6/10 (60%) cases was conducted on extranodal and 19/30 (63%) cases on nodal tissue samples. The remaining 15 cases which could not be categorized by FCI included Hodgkin lymphoma (6 cases), inadequate cellularity (5 cases), Tuberculosis (2 cases), ALCCL (1 case), Mantle cell lymphoma (1 case) and Ewing’s/PNET (1 case). Combining FCI with cytological findings definite diagnosis could be found in 33/40 (82%) compared to 30/40 (75%) in extranodal vs nodal samples. Our data also showed a link between a activated environment (CD38+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The proactivity of FC T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target

**PB2077**

**TREG CD4 PHENOTYPE IN THE PERIPHERAL BLOOD OF LYMPHOMAS**

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**Background:** The regulatory (Treg) cells down-regulate antitumor responses by several distinct mechanisms. One is the adenosinergic pathway, which, through ectonucleotidases, sequentially converts ATP to AMP and generates adenosine, an 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 stromal cells in Hodgkin lymphoma (HL), and B-NHL we analyzed by FC the PB of HL at diagnosis and possibly to distinguish them from those of B Non-Hodgkin lymphomas (B-NHL).

**Methods:** We have analysed by FC the PB of 16 healthy controls (HC), 10 HL and 22 NHL testing within T CD4 cells the expression of CD26, CD38 and CD39.

**Results:** In CD4 T HL, CD39 is expressed in 44% of the subset and the increased expressed (50%) of CD4+CD26+CD38+ 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 (CD38+) and a high CD39, which, in addition to a low CD26, could enhance the generation of adenosine and, therefore, an increased immune suppressive activity. The proactivity of FC T infiltrating can characterize lymphomas in its environment indicating also signals and biological mechanisms representative of possible therapeutic target
the clone of B lymphocytes involved in cancer. This may support that leukemic cells may contribute to create an environment to facilitate immune escape mechanisms. FC analysis of CD26 and CD39, markers likely connected with the adenosinergic pathway, in PB2 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 dis-

ease in terms of clinical behaviour, morphology, phenotype and genetics. Gene expression profiling has made a distinction between two entities germinal center B-phenotype (GC), activated B-center phenotype (ABC). Use of immunohistochemical algorithms for identification of these phenotypes has been translated into clinically feasible approach defining groups as GCB, non-

GCB. These algorithms do not provide completely accurate prognostic infor-

mation so the International Prognostic Index (IPI) which identifies poor-

group status and non-GCB diffuse large B cell lymphoma (DLBCL) is still part of all current diagnostic guidelines; however, the majority of patients have an inter-

mediate 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, bio-

chemical parameters of disease have been subsequently analyzed with statistic

al methods. The expression of bcl-2, bcl-6, CD10, MUM1 and Ki-67 and of markers 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, MUM1 and Ki-67) according to Hans’s and Muris’s algorithm showed positive correlation with good-risk patients identified by IPI. Multiple regression analysis revealed that in bcl-2, bcl-6, CD10, MUM1 and Ki-67 were independent predictors of poor-risk IPI group of patients, (bcl-6: p=0.0107, Ki-67: 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 variable dependent on the value (IPI) (p=0.0210). The mutual impact of bcl-2, bcl-6, MUM1, Ki-67 is significantly related to poor-risk IPI patients.

Summary/Conclusions: Multiple regression analysis proved impact of biomarkers on IPI. Following this analysis, bcl-2 and Ki-67 are independent predictors of poor-risk IPI group of patients. Sequential addition of bcl-2 expression, Ki-67 and GCB phenotype into the IPI significantly improves risk stratification in DLBCL. These finding can be part of treatment strategies that should be considered in future trials.

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 dif-

fuse large B-cell lymphoma (DLBCL) and classical Hodgkin lymphoma (CHL) 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 distinctive histologic or immunohistochemical findings to better differentiate PMLBCL and CHL of the mediastinum

Methods: A total of 32 cases of mediastinal B-cell lymphomas consisting of PMLBCL (N=16), CHL (N=13), and gray zone lymphomas (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, PATA3 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). PATA3 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 PATA3 in CHL and reticular fibrosis in PMLBCL.

PB2080

CASTLEMAN’S DISEASE: HISTOLOGICAL SUBTYPES AND MICROVESSEL DENSITY

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Background: Castleman’s disease (CD) is a rare non-clonal lymphoprolifer-

ative disorder. Most of the cases are characterized by increased vascularity in the affected tissue. The disease falls into two major histological variants: plasma cell type and hyaline vascular type. However, the correlation between microves-

sel density and the subtype of the disease has not been established yet. In this study, we investigate the association between microvessel density and histo-

logical type of CD.

Methods: Twenty-eight lymph nodes from patients diagnosed with CD were used for the study. The age of the patients ranged from 24 to 65 years, 14 were male and 14 were female. Three nodes without evidence of metastasis removed for breast cancer were used as controls. The diagnosis of hyaline vascular CD was based on overall preserved immunearchitecture with typical angio-follicular hyperplasia, circular arrangement of mantle cells around hyalin-

ized germinal centers (“onion skin” pattern). The plasma cell type of CD was confirmed by presence of perifollicular sheets of CD138+ plasma cells. Vessels were identified with CD34 antibodies. Slides were scanned by the whole slide high definition Panoramic scanner. Percentage of blood vessel area (vessel density index) was calculated using Panoramic Viewer software, statistical analysis was conducted with Student’s t-test.

Results: The plasma cell variant of CD was diagnosed in 8 patients, the hyaline vascular variant – in 20 patients. In control group vessels occupied 12±1.5% 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±1.5% (p<0.05) and did not differ from levels in patients with plasma cell variant.

Summary/Conclusions: The highest index of vessel density in the lymph node variant of CD was observed in patients with plasma cell variant. In hyaline vascular variant, the index was characterized by significant variability, which could reflect the heterogeneity of this type of the disease. Increased density of blood vessels in the lymphoid tissue may be considered as a possible target for angiogenesis inhibitors, especially in patients with progressive disease.

PB2081

PROGNOSTIC SIGNIFICANCE OF IMMUNOHISTOCHEMICAL MARKERS IN R-CHOP TREATED DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS

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Background: Despite its clinical, morphological and molecular heterogeneity, diffuse large B-cell lymphoma (DLBCL) is the most frequent lymphoid malign
nancy in adults. The role of immunophenotype variability for the therapeutic outcome has long been the cornerstone for DLBCL management strategy.

Aims: To evaluate the immunophenotypic characteristics of DLBCL and the prognostic significance of specific biomarkers such as bcl2, bcl6, CD 10 and MUM1, in a population-based cohort of patients treated with R-CHOP.

Methods: We performed a retrospective assessment of all cases of DLBCL diagnosed at our institution between 2005-2013. The immunohistochemical expression patterns of all DLBCL patients were analyzed and correlated with the therapeutic response to R-CHOP regimen.

Results: The study included 101 patients diagnosed with DLBCL, with a median age at diagnosis of 57.1 years (19-90 years) and male/female ratio of 1.3/1. Ninety-one patients were eligible for R-CHOP treatment. The median follow-up was 41 months. Out of the 90 cases analyzed by immunohistochemistry CD 10, BCL2, BCL6 and MUM1 expression was found in 17.6%, 50.5%, 72.7% and 81.8% of cases, respectively. Negative expression for CD10, as well as positive expression of BCL2, as 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, hypertriglycerideremia and/or coagulopathy. Histiocyte Society (HS) criteria have been applied for diagnosing HLH, however not all of them are usually showed at the presentation. This disease can be described in two different scenarios: primary (usually in children, genetic, and known as familial form) and secondary (acquired). It can be triggered by a large variety of events that disrupt immune homeostasis. When we talk about triggers, we can divide them in two broad categories, those that cause immune activation and those that lead to immune deficiency. Lymphoid neoplasms can be both.

Aims: Due to the lack of publications about HLH secondary to Lymphoid Neoplasms (LN), we would like to analyze the casuistry of our hospital and making a comparison with the current literature.

Methods: We conducted a retrospective analysis through medical files of all patients with suspected diagnosis of HLH between 1994 and 2017 in our in patient 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 o 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: The creation of these new networks allows strengthening the links between the various actors involved in the field to improve care and answer transversal questions. In this way, MaRIH piloted concerted actions to all its members around immunohematological rare diseases by: 1- increasing the visibility of the actors on the web or during events. The MaRIH website includes all the informations of the members as well as recommendations and events (www.marih.fr), 2- communication and training. MaRIH organizes two annual events, one for patients and another one for professionals. Moreover, MaRIH sends clinical cases by email to professionals and produce an annual webcast, 3- pushing forward research development and epidemiological surveillance. Thanks to his research project manager, MaRIH facilitates the submission and the set-up of new registries or clinical studies. In the future, MaRIH will continue and futher develop all these actions, in close collaboration with the French Ministry of health.

PB2084

CLINICAL FEATURES AND ETIOLOGY OF PATIENTS WITH THROMBOTIC MICROANGIOPATHIES

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Background: Thrombotic microangiopathy (TMA) is a heterogeneous group of disease that has a fatal pattern of endothelial damage. TMA can be found in association with diverse clinical conditions such as carcinoma metastasis, malignant hypertension, infections, and TTP (thrombotic thrombocytopenic purpura). TTP is a rare, life-threatening multisystem disease, characterized by microangiopathic hemolytic anemia, thrombocytopenia, fever, renal dysfunction, and neurological disorders.

Aims: The purpose of this study is to evaluate the etiology associated with TMA.

Methods: All the six TMA patients who were newly admitted to our clinic in two months period were enrolled in this study. Effectiveness, response, adverse effect and safety of plasmapheresis were evaluated using laboratory and clinical findings. (See Table 1).

Results: First patient presented with cachexia, thrombocytopenia, and TMA. He did not respond to plasmapheresis and corticosteroid treatment. We diagnosed carcinoma metastasis and liver metastasis, respectively, through bone marrow biopsy and PET (positron emission tomography). We thus ascertained that TMA was due to carcinoma unknown primary. The second patient presented with general neurological findings like Guillain-Barre Syndrome and paraplegia with renal failure, thrombocytopenia, and TMA. After PLEX and corticosteroid treatment, laboratory and neurological clinical recovery were observed after one month. The third patient had chronic obstructive pulmonary disease and pneumonia in anamnesis. who presented with anemia, thrombocytopenia, fever and pneumonia findings. We conducted PLEX therapy. On the 8th day of PLEX, the patient had anaphylaxis, we performed cardio pulmonary resuscitation. The fourth patient...
presented with acute renal failure with malign hypertension. We performed hemodialysis together with PLEX treatment. Because his diagnosis was acute renal failure, malign hypertension, and TMA. The fifth patient presented with epis-taxis and sepsis. He had chronic TTP diagnosis from two years ago. We diag-nosed the patient as relapse TTP. Early treatment against infection and PLEX increased his platelet counts as early as the second day of treatment. The sixth patient presented with 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% schistoctyes.

Table 1.

| Summary/Conclusions: We diagnosed our first patient with carcinoma unknown primary, who did not respond to PLEX and corticosteroid treatment. The results we received for that patient indicate that PLEX with corticosteroid treatment alone, remain ineffective in cancer-related TMA patients. Etiology of our second patients TMA was idiopathic. His clinical and laboratory findings improved rapidly in response to PLEX and pulse corticosteroid treatment. One viral infection induced TMA patient had anaphylactic reaction receiving his 8th PLEX. Allergic reactions should always be kept in mind when administering PLEX. One patient with TMA and malign hypertension-induced renal failure was successfully treated with PLEX, hemodialysis and antihypertensive treatment. We successfully treated our bacterial infection and sepsis-induced TMA patients with PLEX and antibiotic administration. In second TMA patient, we coupled PLEX with high dosage corticosteroid treatment even though he had an infection. For he had high histocyte count and atypical neurological find-ings.ADMATS 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 TMA is difficult. Etiology, clinical features, laboratory findings should all be taken into account when diagnosing TMA. While it is established that ADAM TS13 defi-ciency is the major cause in acquired TTP, finding the etiology of other TMA is determinant for a successful treatment of the latter. |

PB2085

HAEMOYSIS AS SCREENING TEST IN LYSSOMAL STORAGE DISEASES

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Background: Lysosomal storage disorders (LSDs) are a group of rare inherited metabolic diseases, whose clinical hallmark is organomegaly among others, due to progressive accumulation of several non-catalyzed products inside the lysosomes. This storage leads to intracellular oxidative stress status triggering oxidized metabolites production as oxyesters, which are related to apoptosis and cellular eritosis, as well as haemolysis dysregulation.

Aims: To evaluate the link between LSDs and haemolysis and if it could be used as a screening test in LSDs.

Methods: The osmotic resistance test (ORT) was evaluated in 150 samples including controls, LSDs carriers (LSDs-C) and LSDs patients (LSDs-P). Briefly, the blood was mixed with different concentrations of sodium chloride solution (NaCl) and the haemoglobin released was quantified by spectrophotometry. The raw data was normalized using isotonic solution (0.9% NaCl). The statistical analysis (non-parametric tests and ROC curves), was computed by IBM SPSS statistics v22 software and all statistical tests will be considered and taken as a local 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) whereas no significant dif-ferences were observed between LSDs-C and LSDs-P (p-value>0.05).

Summary/Conclusions: According to our results the ORT test is an useful screening test in LSDs.

PB2086

CLINICAL SIGNIFICANCE OF ELEVATED SERUM COBALAMIN (VITAMIN B12) LEVELS

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Background: Hypercobalaminemia is a frequent but underestimated abnor-mality. Elevated serum cobalamin levels may be a sign of a wide range of dis-eases like solid neoplasms, hematological disorders like myeloproliferative dis-orders, chronic myelogenous leukemia, promyelocytic leukemia, polycythe-mia vera, hypereosinophilic syndrome as well as liver and kidney dis-eases. The analysis shown that haemolysis at 0.48% of NaCl allow us to sort out controls vs LSDs-C/LSDs-P (AUC=0.729) whereas no significant dif-

Aims: We aimed to evaluate the underlying disorders of the patients with high cobalamin levels (>1000 pmol/l) between 01.02.2016-01.02.2017 in Hacette- pe University Pediatric Hematology Department.

Methods: We investigated the patient records of the patients examined between 01.02.2016-01.02.2017 in our department and included the patients with serum cobalamin levels higher than 1000 pmol/l. We excluded the patients who are taking Vitamin B12 supplement.

Results: There were 46 patients with serum cobalamin levels higher than 1000 pmol/l out of 14367 patients seen between 01.02.2016- 01.02.2017 in our department. The reason to check the cobalamin levels were anemia, neu-tropenia and thrombocytopenia in most of the patients. Only 2 patients were referred to our department because of hypercobalaminemia. The underlying disorders were found to be leukemia in 3 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, FMD (familial mediterrenian fever) in 1 patient, chronic kidney failure in 2 patients, sickle cell anemia in 1 patient, factor deficiency in 1 patient, myelodysplastic syndrome in 1 patient, aplastic anemia in 1 patient. Cobalamin excess in 2 patients.

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.
HEMATOTOXIC EFFECTS OF GENERIC TRIAZOLE FUNGICIDES: TEBUCONAZOLE ON WISTAR HANNOVER RATS

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Background: Pesticides are extensively used in agriculture today. Fungicides based on derivatives of triazole are the most widespread all over the world. Tebuconazole (TB) is one of the most frequently used substance of this group. Literature review confirms that triazole fungicides have the ability to cause different hematotoxic effects.

Aims: Since 2007-2016 years we have investigated 10 test-substances of generic tebuconazoles (purity up to 97%) from different manufacturers with purpose to assess their hematotoxic action on male Wistar Han rats peripheral blood in the subchronic 90-days oral toxicity study (according to SOP and OECD 408 recommendations in compliance with GLP).

Methods: The Wistar Han males were randomly allotted to four groups. The input controls of peripheral blood parameters were conducted after a period of animals acclimatization. The goal was to evaluate the physiological state of the Wistar Han rats and the blood picture before rats treatment. Donor’s blood 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), reticulocytes (Retic), 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 change of HGB concentration and RBC count on 4th and 9th weeks of exposure. Morphological changes of RBC (anisocytosis) were seen too. It means that generic TBs had anemic effect. In general, changes of hematological parameters were not principally significant and did not differ from control values at 13th weeks of experiments, except two TB’s, which had shown significant decrease of HGB. Also some of generic TBs (tebuconazole) had leukopenia (leukocytosis) of WBC and RBC in peripheral blood. In case of generic pesticides, the presence of impurities can demonstrate various hematotoxic action. Also the adverse effects on peripheral blood of males Wistar Han rats were observed at a dose of 50 mg/kg/bw/day and demonstrated the lesions of red blood. But abovementioned changes were not so clearly expressed. Any adverse hematotoxic effects at 10 mg/kg/bw/day dose were not observed in all studies.

Summary/Conclusions: As a conclusion, due to our results the triazole fungicides generic tebuconazoles have hematotoxic action. They induce anemia in Wistar Han rats and quantitative white blood cells changes. Today it is very important to investigate the hazardous effects of pesticides on the blood system.

PB2089

WHAT WE CAN DO TO MAKE A STANDARDIZATION AND HARMONIZATION OF APTT?

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Background: Transfusions enlarged many kind of complication in patients therefore per-operative coagulation monitoring was suggested before the surgery. Besides, critical patients may need to be transfused after the surgery. Also there are some diseases which had bleeding events in surgical procedure or spontaneously. We should detect these kind of diseases and we should examine the correct measure of active parsel thromboplastin time (APTT) before surgical procedure by detecting the mild or moderate deficiencies of plasma factor levels and by eliminating the lupus anticoagulant from plasma. This was caused to make the importance of APTT reagents.

Aims: We tried to show the importance of APTT reagents and how to reach the correct measure of APTT in this study.

Methods: We are planning to examine 300 patients, 109 of 300 patients were included as APTT levels were calculated ACL-TOX analyzer by using three different reagents.First reagent was HemosilsAPTT-SP which was sensitive against both plasma factors and lupus anticoagulant. It contains mix colloidal silica and normal range of APTT-SP was 25.4-36.9 s. Second reagent was Hemosil SynthAssi-SS which was sensitive against only plasma factors. It contained mix colloidal silica and normal range of APTT-SS was 25.1-38.6 s. The third reagent was Hemosil SynthA-Fox-SS which was sensitive against only lupus anticoagulant. It contains ellagic acid and normal range of APTT-SS was 21.5-30.4 s.

Results: Forty-five of 109 patients had normal level of APTT by measuring three types of reagents. Seventeen of 109 patients had long level of APTT by measuring three types of reagents. Twenty of 109 patients had long level of APTT by measuring Hemosil SynthAssi-SS reagent and had normal level with Hemosil SynthA-Fox-SS and Hemosil APTT-SP. Seven of 109 patients had long level of APTT by measuring Hemosil SynthAssi-SS reagent and Hemosil APTT-SP reagent and had normal level of APTT with Hemosil SynthA-Fox-SS. Twenty of 109 patients had long level of APTT by measuring Hemosil SynthA-Fox-SS and Hemosil SynthAssi-SS reagent and had normal level of APTT with Hemosil APTT-SP.

Summary/Conclusions: Haemostasis is a complex physiological cascade which was began at the endothelium injury. Many kinds of complex procedures occurred with purpose to stopping bleeding. Because of this complex cascade pathway, bleeding is not occurred every kind of APTT level. Bleeding events usually occurred at <30 level of plasma factors but mild or moderate plasma factor levels can caused bleeding. Therefore sensitivity of APTT reagents are very important. Every kind of APTT reagent do not have equal sensitivity against plasma factor levels, phospholipid composition and concentration in thromboplastin reagents. Several studies suggested that range of APTT should be determine according to the devices and reagents and also several studies compared APTT reagents which was included silica, ellagic acid and phospholipids by composed of synthetic or animal orginated. We need further studies to make a standardization and harmonization of APTT.
Summary/Conclusions: Immunosuppressive therapy including cyclosporine with or without steroid has been reported as the most effective treatment to primary acquired PRCA. Consistently, we had a dramatic response to immuno-suppressive 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 included 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 bone marrow severe aplasia, 2 (22.2%) had severe and 4 (44.5%) had very severe aplastic anemia. All of the patients received immunosuppressive therapy (consisting of antithymocyte globulin, cyclosporine A and steroids), 2 remained transfusion independent, 4 underwent bone marrow transplantation- 2 from a matched related donor and 2 from a matched unrelated donor. One patient with refractory disease received, as an alternative first line therapy, eltrombopag. Complete response was achieved in 22.2%, partial response was achieved in 22.2%, relapse occurred in 11.1% and 44.5% of the patients had refractory disease. The overall survival was 77.8%.

Summary/Conclusions: A remarkable progress has been made during the past decades in the understanding of pathogenesis and management of children with aplastic anemia. Bone marrow transplantation from a matched related donor is the recommended first line therapy resulting in an excellent survival rate that exceeds 90%. In the future the development of targeted strategies for patients with refractory 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 chronic gastrointestinal blood accounts for the majority.

Aims: Of the study is to evaluate retrospectively adult men with IDA that were hospitalized in our Hematology Clinic.

Methods: Two hundred fifteen male with IDA were enlisted at this study from January 2005 to december 2015. Anemia was defined as Hg <13g/dL using the WHO criteria. IDA was considered present if serum ferritin was 15 ng/mL combined with serum iron concentration <30ug/dL with a transferrin saturation of <10%. Complete physical examination, the history of the disease and fecal occult blood test (FOBT) of three spontaneously passed stools was done in all patients. All patients had complete blood count, serum and total iron binding capacity, and a serum ferritin level. Most patients underwent esophagogastroduodenoscopy (EGD). Colonoscopy was performed if lesion that caused IDA was not found, and/or FOBT was positive. The abdominal CT scan were performed according to clinician’s recomandation together with other tests related with blood lost.

Results: The median age was 62 ( range 32 to 86 ) years old. 168 of 215 ( 78.5%) males with IDA had symptoms such as fatigue, dizziness, or digestive complaints. The history of prior gastrectomy, hemorhoid, 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 under-went EGD. The most common findings from EGD were gastritis (48 patients) and peptic ulcer (39 patients). Seventy eight (36.27%) patients were found to have upper gastrointestinal disorders ( 20 patients with erosive gastritis, 19 gastric ulcer, 16 duodenal ulcer, 23 gastric cancer. Eighty-nine (41.39%) patients underwent colonoscopy. That showed 44 clinically important lesions that probably caused IDA; colon cancer in 17 (7.90%) patients, colon polyp in 10 (4.65%) patients and hemorrhoid in 17 (7.90%) patients. Concerning malignant lesions which are responsible for IDA, the malignant lesions were found more frequent in patients older than 50 years accounting for 20.45% (27/132 patients) and patients younger than 50 years 17.80% (13/73 patients).

Summary/Conclusions: This study demonstrated that gastrointestinal blood loss is the main cause of IDA in adult men, and that there is a high rate of malignancy in men older than 50 years.

PB2093
IMPACTS OF CLINICAL AND BIOCHEMICAL PARAMETERS ON KEY HEMATOLOGICAL INDICES IN ADULTS: A COHORT STUDY
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Background: Studies in Caucasians have shown that values of hematological parameters could be affected by a wide variety of factors, most notably age and gender. However, parallel work in different ethnic populations, especially from Asia-Pacific region, is lacking. Importantly, it remains largely unknown whether some fundamental variables such as nutritional status, lipid profile, and hepatitis infection (either HBV or HCV) also affect the variation of values in hemogram.

Aims: Therefore, we conceptualize this study to explore through several key parameters regarding their potential impacts on the changes of hemogram.

Methods: Adult individuals aged 18 or older from several adjacent villages in Yun-Lin County, located in the central part of western Taiwan, who came to our hospital for annual health exams were screened for the current study. The work, retrospective in nature, was approved by institutional IRB. Information on age, gender, hemogram, levels of total cholesterol (TC), triglyceride (TG), apolipoprotein B (Apo B) as well as albumin, and results of serological testing for hepatitis B and C infection, was obtained from a centralized digital data base. All the clinical data, after given a coding number for each case, were encrypted and provided to the investigators without identifiable personal information. We analyzed the impacts of various parameters on several key hematological indices.

Results: Overall, 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 = 0.82211, p <0.0001, Pearson’s correlation), we did not incorporate TC in our multi-variate analysis. Several key variables were found to influence some hematological indices in the multi-variable regression model. Increasing age and male gender negatively affected the platelet count, whereas higher Apo B level was associated with elevated platelet count. Surprisingly, hepatitis C carriers with normal hepatic function had slightly higher platelet number than non-HCV carriers. Gender and serum albumin level were the major determinants of variation in hemoglobin level. 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.
Apo B, while cellular count, and platelet count all impose risk of thromboembolism, further work exploring the interactions and impacts of these parameters on the development of cardiovascular diseases should be mandatory.

PB2094

UNUSUAL DISTRIBUTION OF INTERLEUKIN-10 C-592A GENE POLYMORPHISM IN PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA FROM NORTH-WESTERN RUSSIA

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Background: Primary immune thrombocytopenia (ITP) is a rare hematological disease with unknown etiology. It is characterized by heterogeneity of the laboratory parameters as well as the features of clinical manifestation. DNA polymorphism of several cytokine genes has been suggested to modulate the risk of ITP development or/and treatment response in distinct population groups. There is no data on the prevalence of cytokine gene polymorphisms in ITP patients from the North-Western region of Russia (NWR).

Aims: To establish the features of genotypes distribution for several cytokine promoter gene polymorphisms in ITP patients from NWR.

Methods: A total of 68 patients (59 women and 9 men) with chronic primary ITP were involved in the study. The median age of the group was 57 years (range: 24-77). The mean duration of ITP was 7 years (2-48). In 19 (32.2%) women, ITP was diagnosed before 30 years old; 26 (38.2%) patients (5 men and 21 women) were diagnosed at age 30-50 years; 23 (33.8%) patients (13 men and 19 women) developed ITP after 50 years old. The control group consisted of 240 healthy persons originated from NWR. Nucleotide variations in the genes coding for interleukin (IL)-1b (-31T/C), IL-6 (-174G/C), IL-10 (-592A/G) and tumor-necrosis factor alpha (TNFA -308G/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 person of who had IL-10 -592AA 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 (15.8% vs 8.2% respectively; OR=2.1, 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 -592AA 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-dependent 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 corticosteroid-dependence. We 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 10mg/kg/week. Patients have been evaluated every week until platelets were higher than 30x10^9/L for at least 2 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 polyspecific immunoglobulins (Ig), cyclosporine, methotrexate and/or romiplostim. 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 platelet counts 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 types of response were: • One patient did not respond after 60 days, and we maintained for 7 months in the absence of active treatment. The combined was necessary during 6 months. • 2 CRs still undergoing combined dose reduction (current dose romiplostim 2mg/kg/week and azathioprine 50mg id). 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-dependence and thrombopoietin analogs alone. More studies are needed to clarify the mechanism of complementation between the two drugs.

PB2096

AGONIST-INDUCED PLATELET REACTIVITY CORRELATES WITH BLEEDING IN HEMATO-ONCOLOGICAL PATIENTS

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Background: Prophylactic platelet transfusions are administered to prevent bleeding in hematologic-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 help 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 hematopoietic-oncology patients aged 18 years and above after written informed consent. Furthermore, an expected need for platelet transfusions was necessary. Bleeding was graded according to the WHO bleeding scale. Platelet reactivity to stimulation by either adenose diphosphate (ADP), crosslinked-collagen-related peptide (CRP-α,CRP-β), PAR-1-activating peptide and PAR-4-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 0.23 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-α; 0.59 [0.37-0.94] for PAR-1-AP and 0.43 [0.23-0.79] for PAR-4-AP. The platelet count was not correlated with bleeding (OR 0.99 [0.96-1.02]).

Summary/Conclusions: The examined-induced platelet reactivity was significantly correlated to bleeding. Platelet function testing could provide a basis for a personalized transfusion regimen, in which platelet transfusions are limited to those at risk of bleeding.

PB2097
TUMOR NECROSIS FACTOR-A AND TUMOR NECROSIS FACTOR-B SINGLE NUCLEOTIDE POLYMORPHISM AND CHRONICITY IN EGYPTIAN PEDIATRIC PATIENTS WITH IMMUNE THROMBOCYTOPENIA
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Background: Although the etiology of immune thrombocytopenic purpura (ITP) remains unclear, both genetic and environmental factors may contribute to the development of disease. Tumor necrosis factor alpha & beta (TNF-α and TNF-β) are 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. In the case of the disease might be the base for future specific immunomodulatory therapies for chronic ITP (cITP) in children.

Aims: The current case-control study aimed at detecting TNF-α (-308 G/A) and TNF-β (-252 A/G) genes polymorphism in Egyptian children with cITP and studying their possible association with chronic evolution of the disease.

Methods: The current study included 80 Egyptian cITP patients at Pediatric Hematology Unit, Cairo University (mean age 7.08±3.64 years) and 100 matched unrelated healthy controls. Genotyping was performed using polymerase chain reaction restriction fragment length polymorphism technique (PCR-RFLP).

Results: TNF-α genotyping revealed that wild G/G, heterozygous A/G and homozygous A/A genotypes among cITP patients were 81.2%, 15% and 3.8% respectively versus 79%, 20% and 1% in control group, while TNF-β wild A/A, heterozygous A/G and homozygous G/G genotypes among cITP patients were 55%, 40% and 5% respectively versus 60%, 28% and 12% in control group, with no statistically significant difference between both groups. Patients having homozygous TNF-α genotype showed statistically significant higher mean age, longer disease duration & lower mean platelet count (p=0.005, 0.024 and 0.008 respectively). TNF-α polymorphism was more frequent among unresponsive patients compared to responsive patients with statistically significant difference. Calculated risk estimation revealed that combined genes polymorphism conferred three fold increased risk of development of cITP (OR=3.491, 95% CI: 1.235-9.869, p=0.015).

Summary/Conclusions: We hereby report a strong association between combined polymorphisms of both TNF-α & TNF-β genes and susceptibility to chronicity of ITP in Egyptian children. Further studies for gene polymorphisms which could affect the pathogenesis of ITP and facilitate the development of new therapeutic modalities are recommended.

PB2098
PROGNOSTIC FACTORS IN PRIMARY IMMUNE THROMBOCYTOPENIA OF CHILDHOOD
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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. Local 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 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 10x10^9/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=0.01) and received more often intravenous gamma globulin and/or corticosteroids (p>0.05). None of the children exhibited severe spontaneous bleeding.

Summary/Conclusions: Even though ITP in children is usually a self-limited disease, with rare serious bleeding complications, the newly diagnosed/persistent and the chronic form of the disease are characterized by different predictive parameters that can be used in clinical practice.

PB2099
CANCER-ASSOCIATED IMMUNE THROMBOCYTOPENIA
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Background: Cases of cancer-associated immune thrombocytopenia (IT) have been reported recently, but there are few reports and case series that describe clinical features and response to treatment.

Aims: We report our experience of 10 years at a single hospital in Spain, in patients with IT concurrent with neoplasia.

Methods: We identified the patients by data search of hospital records from 2006 to 2016, with diagnosis of IT with previous diagnosis of cancer, not related with chemotherapy or radiotherapy, not suggestive of bone marrow infiltration, drug-induced, infection of disseminated intravascular coagulation. For the diagnosis, the examination of bone marrow was not mandatory.

Results: The two most common cancers associated with IT were bladder and lung neoplasms, but the occurrence of prior cancer (third part of patients) was not uncommon. The IT can appear at any stages of cancer, and it is mainly detected at the first two years after the diagnosis when the patient have been in acceptable antitumoral response. They usually manifest with very low platelet count <20,000, but not always with evident clinical bleeding. The response to therapy was fast and complete with corticoids (usually in the first week) in the majority of patients, but some cases require the combination second line with immunoglobulins or thrombopoietin receptor agonists, and in the follow-up, the response was persistent without recurrence in the first year post-treatment (Table 1).

Table 1.

Summary/Conclusions: The CAIT is a rare hematological paraneoplastic syndrome that occur in solid tumors, usually associated to low platelet count but without life-threatening bleeding, requiring therapy with corticosteroids as first line, and generally related with a benign clinical course with a rapid and persistent response.

PB2100
THE ROLE OF MEAN PLATELET VOLUME IN NEONATAL SEPSIS: A META-Retrospective CASE CONTROL STUDY IN A LEVEL III NEONATAL INTENSIVE CARE UNIT
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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 two groups: neonates with clinical sepsis (as defined by either culture positivity and/or clinical features plus treatment with antibiotics exceeding 48 hours) and 75 healthy controls (as defined by neonates in whom antibiotics were never started or discontinued when cultures were negative for 48 hours and the absence of clinical features of sepsis). Total white blood cell count, C-reactive protein, immature to total neutrophil count and mean platelet volume between neonates with sepsis and healthy controls. C-reactive protein and immature to total neutrophil count (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. Here, we compared the two groups.

Results: There was no statistically significant difference in the mean platelet volume between the study group and the control group (p value 0.9 in the first 24 hours and p value 0.9 in the second 24-48 hours). There was however, a statistically significant difference between immature to total neutrophil count and C-reactive protein on both samples (p value <0.0001) (Table 1).

Table 1.

Summary/Conclusions: In our study there was no statistically significant difference in the mean platelet volume values between neonates with sepsis and healthy controls. C-reactive protein and immature to total neutrophil count continue to be reliable markers of neonatal sepsis.

PB2101

IS PLATELET TRANSFUSION WARRANTED IN PATIENTS WITH ACUTE TTP REQUIRING CENTRAL VENOUS CATHETER INSERTION?

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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, the rates of 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. A total of 48 patients confirmed to have TTP with an ADAMTS13 <5% underwent line insertion: 94 central venous catheter lines were inserted: 40% femoral, 60%-internal jugular vein. The median number of lines inserted per patient episode was 3, with a range of 1-5. Median presenting platelet count for first line insertion was 25x10^9/L (IQR 9-26 x10^9/L). 70% of lines were inserted by critical care and the remaining 30% by interventional radiology. Platelet transfusion was not administered pre line insertion and no 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 thromboticocytic 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

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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 agonists.

Methods: Treatment with thrombopoietin agonists was initiated in 20 adult patients (pts) with refractory cITP between June 2011-2016. Patients resistant or intolerant to the first agonist switched to the second one. Complete response (CR) was defined as a platelet count of ≥100x10^9/L.

Results: Eltrombopag was administered in 15 pts, 6 male-9 female with a median age of 46 years (19-76 yrs) for 13 months (1.4-54 mn). 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 (5-450 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 63 years (33-70 yrs) for 15 months (6-77 months). 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 (9-140 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 value=0.01). The BsmI polymorphism B allele was higher in ITP group than that in controls but in statistically insignificant difference ($\chi^2=2.125$, P=0.145). Bb genotype played a protective role in ITP incidence.

**Summary/Conclusions:** This is the first published report on VDR gene polymorphisms in adult primary ITP patients. The BsmI genotype was associated with increased risk for ITP incidence with no obvious effect on bleeding severity, platelet count nor site of bleeding.

PB2104

**A SURVEY OF THE TREATMENT OF THE PREVENTION OF NAIT IN THE UK AND IRELAND**

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**Background:** Neonatal alloimmune thrombocytopenia, (NAIT) is caused by maternal antibodies generated against alloantigens carried on fetal platelets, which cross the placenta and induce destruction of platelets in the fetus. In most cases the maternal immunisation is triggered by exposure to fetal blood at delivery. As a result, the clinical presentation tends to be more severe in subsequent pregnancies. Recent studies and guidelines have suggested that intravenous immunoglobulin (IVIG) with or without steroids can significantly reduce the severity of thrombocytopenia in subsequent pregnancies.

**Aims:** We set out to establish if there is consistency in the management of the prevention of NAIT across Ireland and the United Kingdom (UK).

**Methods:** A survey was set up on Survey Monkey and all members of the UK-Ireland Haematology group were contacted by email with a link to the survey in January 2015. In total 90 individual Specialists were contacted across 70 centres.

**Results:** 30 responses were received to the following questions. Who manages the prevention of NAIT in your centre? 34% of respondents stated that it was managed jointly by haematologist/fteto-maternal specialists, with 26% responding it was overseen solely by haematologists and 40% solely by feto-maternal specialists. Secondly what risk stratification each respondent used to decide risk of NAIT in the current pregnancy? 82% stated that they took into account multiple risk factors but 18% stratified risk based only on the outcome of previous pregnancy. Thirdly how many groups do you define after risk stratification? 60% identified 3 strata of risk (standard, high and very high) with 40% classifying two risk groups (standard versus high risk). Fourthly respondents outlined their management of a standard risk group defined as confirmed thrombocytopenia with antibody. 43% give IVIG 1g/kg weekly from 20 weeks, 28% give 1g/kg from 20 weeks increasing to 2g/kg at 32 weeks, 32% weeks starting 1g/kg with increasing 0.5g/kg weekly. 23% referred to feto-maternal specialist to decide IVIG. Just 20% give 0.5g/kg of steroids from 20 or 32 weeks. For high risk pregnancies defined as confirmed antibody positive with previous intracranial haemorrhage (ICH) after 28 weeks: 36% of centres give IVIG 1g/kg from 20 weeks, 36% give 1g/kg from 28 weeks increasing to 2g/kg at 32 weeks with 14% giving 2g/kg from 20 weeks and 14% initiating at 12 weeks. 40% gave 0.5g/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, 6% plan a caesarean section but with no set time and 16% have no specific protocol for delivery.

**Summary/Conclusions:** The results of this survey reveal that the optimal medical management for the prevention of NAIT, namely the medication, dosages 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.

PB2105

**THE EVALUATION OF REACTIVE OXYGEN SPECIES IN CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA AND HELICOBACTER PYLORI INFECTION VERSUS CHRONIC ITP WITHOUT HELICOBACTER PYLORI INFECTION**

M. A. García, M. Vázquez-Delgado, M. Garmán

**Background:** Chronic idiopathic thrombocytopenic purpura (ITP) is an acquired disease characterized by a low platelet count caused by an immunological peripheral platelet destruction or a decreased platelet production. Several studies have shown increased reactive oxygen species (ROS) levels in chronic ITP and also a possible association between Helicobacter pylori (H. pylori) infection and immunological peripheral platelet destruction.

**Aims:** To evaluate whether patients with chronic ITP and H. pylori infection exhibit higher ROS levels compared to patients with chronic ITP and no H. pylori infection and whether there are statistically significant differences between the two groups.

**Methods:** We studied 29 patients with chronic ITP (median age 39 years) hospitalized in the Clinic of Hematology, Filantropia City Hospital, Craiova, Romania, between 2014 and 2016 (informed consent obtained). All patients were diagnosed with ITP, other causes of thrombocytopenia having been ruled out by bone marrow aspiration. The patients were divided in two groups: patients with ITP and H. pylori infection (group A) and patients with chronic ITP without H. pylori infection (group B). Both groups were used to identify the presence of a H. pylori infection and reactive oxygen species were evaluated by FORT (Free Oxygen Radicals testing) test from a single drop of capillary blood, at the time of diagnosis, before the administration of any drug (the normal value of FORT is considered less than 2.3 mmol/L H2O2). However, statistically significant differences were found in favour of group A, with higher ROS values than group B. The A group also associated lower platelet counts and more patients pertaining to this group relapsed in comparison to group B.

**Summary/Conclusions:** In chronic ITP, increased levels of ROS are associated with elevated autoantibody production. Autoantibodies are involved in platelet destruction via highly a immunomnengenic activity. On the other hand, association of H. pylori infection, via chronic inflammation, led to a supplementary increase in ROS levels and increased platelet destruction.

PB2106

**IMMUNE THROMBOCYTOPENIA AND PREGNANCY: A SPANISH CASE SERIES OF 270 PREGNANCIES IN PRIMARY ITP**


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**Background:** Effect of pregnancy on the course of primary immune thrombocytopenia (ITP) is not well known. Besides, due to the lack of clinical assays, it is difficult to predict outcome predictors of pregnancies and neonates born to mothers with ITP is scarce.

**Aims:** To evaluate management and results of pregnancy and delivery on pregnant ITP women and on their offspring.

**Methods:** All women diagnosed of primary ITP (according to international consensus criteria) from 2011 to 2016 in 23 Spanish Hematology Departments who had at least one pregnancy after ITP onset were included in this registry.

**Results:** We included 270 primary ITP pregnancies from 184 women. At pregnancy diagnosis, we observed a majority of chronic ITP cases (71.4%). At ITP diagnosis, 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%). Primary 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-haematostatic 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, 70-181). 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 underlying etiology cannot be identified and secondary when a certain etiology exists. Data concerning ITP characteristics at a national level are limited.

Aims: The purpose of the study was to access systematically the demographic, clinical, laboratory and treatment-related data of ITP in Greece based on the national database (ITP registry) operated and supported by the Hellenic Society of Haematology.

Methods: Patient data were collected over 2013-2016. The data source is a unique database initiated and managed by the Haematology Department of the University of Crete (UoC) and supported by the Center of Information and Communications Technologies of the UoC. The registry has been configured for national and regional base usage considering hospitals as the core unit. A central database administrator has access to a platform where he/she can record and study patients’ data. The entire project has been developed using the robust open source tools of operating systems and Relational Data Base Management System (RDBMS) packages.

Results: We analyzed data from 696 adult ITP patients registered from 14 different hospitals from all parts of Greece. The median age at diagnosis was 53 years (range 15-97 years). Two peaks were observed at the age of 19-30 and 71-80 years. There was a female (60.89%) predominance versus male (39.1%) predominance with higher frequency of females in younger (19-30 years) and of males in older (71-80 years) ages. Females appeared with more severe thrombocytopenia. The majority of patients presenting with hemorrhagic symptoms (70.9%). The main manifestations were cutaneous bleeding (64%), oral cavity bleeding (20.9%), epistaxis (8.9%), menorrhagia (7.8%) and gastrointestinal bleeding (5.5%). 430 patients (61.78%) had primary ITP and 266 (38.22%) secondary ITP. Among these secondary ITP cases, 44.2% were related to infectious agents, 25.74% to drugs, 17.17% to collagen vascular diseases and 12.87% to cancer. Patients with positive ANA antibodies with no evidence of any underlying diseases were included in the primary ITP group. The main patient comorbidities were hypertension (22.64%), thyroid disease (12.32%) and cardiovascular disease (10.17%). Treatment was given in less than 50 x 10^9/l (IQR, 36-172). 43 patients (23.4%) experienced 57 bleeding episodes.

The results of the study showed that hemorrhagic symptoms are not frequent during pregnancy. The main manifestations were cutaneous bleeding (74%) and gastrointestinal bleeding (24.97%). The main manifestations were cutaneous bleeding (64%), oral cavity bleeding (20.9%), epistaxis (8.9%), menorrhagia (7.8%) and gastrointestinal bleeding (5.5%). 430 patients (61.78%) had primary ITP and secondary ITP. Among these secondary ITP cases, 44.2% were related to infectious agents, 25.74% to drugs, 17.17% to collagen vascular diseases and 12.87% to cancer. Patients with positive ANA antibodies with no evidence of any underlying diseases were included in the primary ITP group. The main patient comorbidities were hypertension (22.64%), thyroid disease (12.32%) and cardiovascular disease (10.17%). Treatment was given in less than 50 x 10^9/l (IQR, 36-172). 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.
NOVEL TECHNIQUES FOR MONITORING GALNZZMTHROMBASTHENIA 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 Rotational thromboelastography (ROTEM).

Methods: A nine year old female patient with GT underwent teeth extraction. The patient received platelet transfusion around the procedure. Complete blood counts, ROTEM, FC to detect the number of donor platelets and their ADP dependent 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% donor platelets equivalent to 55,620 donor platelets. Platelets activation was determined following ADP addition by examination CD62 antigen expression. Seven days post platelet transfusion FC demonstrated 2.6% equivalent to 8,658 donor platelets.

The decline in the number of active platelets was associated with a reduced clot firmness (MCF) and lower α-angle as assessed by ROTEM (Figure 1).

Figure 1.

Summary/Conclusions: Clinical decision making in patients with GT may be aided by application of novel techniques, evaluating the number of active donor platelets and actual clot formation. This data may help improving monitoring and better patients’ care.

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. Immunohistochemical (IHC) staining using Avidin-Biotin complex system (ABC) was conducted. For IHC, dissections prepared from blocks were taken onto poly-L-lysine coated slides (MicroSlides Snowcoat X-tra, Surgipath, Richmond, IL, USA) and kept in an incubator at 37°C overnight. Dissections were treated with 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 the staining; i.e. negative (0), weak (1+), moderate (2+) and strong (3+) (1). All patients with ITP who had splenectomy were in chronic phase of the disease. The present study was supported as a Scientific Research Project by Adnan Menderes University (TPF-15027).

Results: c-mpl positivity was statistically significant in patient group who did not have splenectomy (Table 1). In patient group who had splenectomy, c-mpl was not associated with refractory status.

Table 1. c-mpl positivity in patients group who had and did not have splenectomy.

Summary/Conclusions: Status of c-mpl in ITP is ambiguous. Significant level of positivity in patient group who did not have splenectomy might be useful for splenectomy indication.
PREDICT REQUIREMENT FOR SECOND LINE THERAPY IN IMMUNE THROMBOCYTOPENIA

Background: Immune thrombocytopenia (ITP) is an acquired, immune-mediated disease that is characterized by increased destruction of platelets by autoantibodies. ITP is characterized by mucocutaneous bleeding. Rarely, life-threatening bleeding such as central nervous system bleeding can occur. Typically, patients have isolated thrombocytopenia. The diagnosis of ITP is one of exclusion. Corticosteroids are chosen as a first-line therapy for adult patients who require treatment. Responses to first line therapy with corticosteroids is about 80% with approximately 20% to 30% long term complete remission. Most patients finally relapse, requiring second-line therapy. Aim: Our aim was to evaluate the potential 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’ thrombocyte counts were below 30 x10^9/L at diagnosis. All patients received initially methylprednisolone (MP) 1 mg/kg/day. For patients who responded with platelet count ≥150 x10^9/L methylprednisolone was tapered over 3 months. Those who were unresponsive to MP or relapsed after a complete response, were treated with second line therapies that splenectomy or medical treatment agents. The platelet counts of the patients on day 0, 3 and 7 were evaluated by complete blood counts and were confirmed with peripheral smear examination. Effect of the platelet counts on day 3 and 7 were compared in terms of second line therapy requirement or not. A platelet count of >30x10^9/L on day 3 and >100x10^9/L on day 7 was considered as a complete response. Vaccination against encapsulated organisms was given and imaging was done to detect accessory spleen before splenectomy. Results: Baseline characteristics of the cohort of 43 patients with an initial diagnosis of ITP are shown in Table 1. The mean age at diagnosis was 51 years (18-84) with female/male: 25/18. All patients presented with severe thrombocytopenia (platelet counts below 30.0 x10^9/L). Most patients presented with mucocutaneous bleeding (n=39), only three patients had genitourinary or gastrointestinal tract bleeding and one patient was asymptomatic. Bone marrow examination and peripheral smears examination confirmed the diagnosis of ITP. Ten patients had mucocutaneous manifestation and aspiration and biopsy was done in 14 (32.6%) patients due to various reasons mainly, failure to respond to ITP treatment (7 patients) and advanced age (7 patients). On third and seventh day of MP therapy, median platelet counts were 30x10^9/L (2.0 x10^9/L-150 x10^9/L) and 100 x10^9/L (1.0 x10^9/L-347 x10^9/L), respectively. A platelet count on third day and seventh day in each patient was evaluated in comparison to patient’s baseline. A significant association was found in correlation analysis (p<0.05). 21 patients (48.8%) required second line therapy which were splenectomy or medical treatment agents. Medical therapy consisted of rituximab, eltrombopag, danazol. There was a statistically significant difference between the patients with platelet count below and over 30x10^9/L on 3rd day of the MP therapy in terms of requirement for a second line therapy. (p=0.04). On the other hand, when 7th day was taken into consideration, there was not a statistically significant difference when cut off was taken at 100 x10^9/L (p=0.09) or 50 x10^9/L (p=0.08). Summary/Conclusions: In the era of novel therapies used as second line, predicting the prognosis of an ITP patient at diagnosis has been a challenge. If disease related factors at diagnosis can be identified, then patients can be rescued from long term corticosteroid exposure and its adverse effects. Early response of platelet counts after starting corticosteroids seems to predict long-term complete remission. Expanding the study population may contribute more to our findings.
PB2115

SWITCH OF TPO-MIMETICS IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: FLORENCE MONOCENTRIC EXPERIENCE

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Background: Primary immune thrombocytopenia (ITP) is an immune-mediated condition characterized by isolated thrombocytopenia, with peripheral blood platelet count <100,000 in the absence of an identifiable underlying cause of thrombocytopenia. Clinical studies in patients with ITP demonstrated that thrombopoietin (TPO) mimetics increase platelet production and can outpace platelet destruction.

Aims: We evaluated patients treated with both TPO-mimetics.

Methods: From November 2008 and February 2017, 65 patients were treated with TPO-mimetics with a median follow-up of 29 months (1-96): 39 patients underwent therapy with Romiplostim and 26 to 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 they 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 was 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 receptor agonist 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 intertribal marriage. Unfortunately, some patients have got more than one autosomal recessive genetic disorder, owing to complex consanguinity which might further complicate proper management plans.

Aim: This report describes a case of combined Glanzmann’s thrombasthenia and MSUD, and to review the existing data of platelet function disorders in Oman.

Methods: Case report and retrospective data analysis of all cases with confirmed or suspected platelet function disorders in Pediatric Hematology Unit, Sultan Qaboos University Hospital, Muscat, Oman from January 2006 till December 2016. Results: A 2 months 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, full blood count and coagulation screen were perfectly normal. Unfortunately, she developed profuse bleeding at the site of G-tube insertion, followed by massive hematemesis. The patient received multiple blood products, but bleeding didn’t 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 aggreometry 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 facitliy to the lack of agreed upon consensus 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 ASPIRIN 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-gregant effect of aspirin and, specially, with clopidogrel has been reported. The VerifyNow® System (Accutronics, San Diego, CA, USA) performs a turbidimetric-based optical detection of induced platelet aggregation in response to major anti-platelet 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) <208 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. 

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 PGE1 to the ADP test may increase its sensitivity. VerifyNow® assay seems to overestimate the effect of clopidogrel, since hyper-response data are not reproduced by other techniques. According to our results, a high interindividual variability in response to clopidogrel is observed.

PB2118
THROMBOPOIETIN-RECEPTOR ANTAGONISTS IN ITP - EXPERIENCE OF A CENTER
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Background: Thrombopoietin-receptor antagonists (TRA), romiplostim and eltrombopag, are part of the treatment of chronic immune thrombocytopenia (ITP), resistant to first line therapy (corticosteroids and/or immunoglobulins) and with a significant bleeding risk. Both are approved for adult patients, but only eltrombopag was approved for pediatric use. When used before splenectomy, these treatments may serve as a bridge for surgery or even postpone/avoid the procedure.

Aims: In this report, we aim to evaluate the response to TRA treatment in patients with ITP and associated side effects in our center.

Methods: Inclusion criteria: patients with ITP resistant to first line treatment. Patients were only included if they were responders to first line therapy (corticosteroids and/or immunoglobulins). Clinical evolution and adverse effects were evaluated by retrospective analysis.

Results: Thirty-eight patients with ITP were included: 31.4% (12) were male and the median age at diagnosis was 38 years. 44.7% (17) had relapsed/resistant disease after splenectomy and 13.2% (5) were treated with a TRA as a bridge for this procedure. Sixteen (42.1%) of ITP patients were treated with romiplostim: 12 patients (75%) had a response to treatment, and 4 (25%) were resistant. In 11 of these patients, romiplostim was replaced by eltrombopag, either because of resistant disease, or more convenient administration (oral therapy). Thirty-three (86.8%) patients were treated with eltrombopag (5 pediatric cases); 27 patients (81.8%) responded while 8 patients had resistant disease (3 of these were HIV positive). The response rate was higher in patients with previous splenectomy (91.7% with romiplostim and 92.9% with eltrombopag) compared to those with no previous splenectomy (25% with romiplostim and 73.7% with eltrombopag). Six patients maintained response after treatment suspension (5 treated with eltrombopag and 1 treated with romiplostim). Generally, both treatments were well tolerated, with only one case of eltrombopag suspension because of a thromboembolic event.

Summary/Conclusions: In the current study, both TRA were effective in the treatment of ITP resistant to several lines of treatment, with similar response rates. As described in the literature, the response rate was higher in patients with previous splenectomy, and some cases maintained response after treatment suspension. The toxicity profile was acceptable. However, there are some concerns about their safety in long term therapy, namely the development of myelofibrosis, cytogenetic abnormalities and malignant evolution. Consequently, both are urgent need for further studies to define the optimal period of treatment and surveillance, especially in pediatric patients. In our center, the median time of treatment with eltrombopag for all patients was 5.5 months (range between 1 to 34 months) and with romiplostim was 12 months (range between 1.5 to 85 months). The duration of treatment with eltrombopag in children and adolescents was around 6 months.

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.

Aims: In hematological evaluation not only platelet (PLT) counts but also 3 important platelet indexes are taken into consideration. In hematological evaluation not only platelet (PLT) counts but also 3 important indexes are taken into consideration. Changes of platelet (PLT) count and platelet index in Turkish preterm and term infants. Platelet counts, and platelet 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 rised again conspicuously between 34th and 37th weeks. At 38th week a dramatic increase of platelet counts were observed. MPV and PDW were kept stable, while MPV values increased dependent on the gestational age. When we classified newborns in groups of 24th-31th, 32nd-36th, and 37th, and 38th weeks, constant, and statistically significant variations were observed between groups.

Summary/Conclusions: 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.
Background: Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by immune-mediated platelet destruction and suppressed platelet production. ITP may occur concurrently or precede the occurrence of SLE, which would have great diagnostic significance. ITP may also be the first early sign of the disease. Few studies have addressed the risk of systemic lupus erythematosus (SLE) after ITP.

Aims: To estimate the risk of SLE after ITP in adult Jordanian patients

Methods: All patients diagnosed with ITP and with a platelet count <100x10^9/L between September 2002 and January 2017 were included in the study. Patients were retrospectively reviewed for diagnosis of SLE, and inclusion criteria included only those patients who had initial ANA screen at the time of the first presentation of ITP. All patients with the diagnosis of SLE at the time and before the presentation of primary ITP were excluded from the study.

Results: This study included a total of 58 patients (43 females and 15 males) who were followed up for a period of 14 years. Their age at the baseline ranged from 16 to 65 years with a mean (SD) of 31.2 (13.3). ANA was positive in 11 (19.0%) patients. Over the period of follow up, 9 (15.5%) patients developed lupus. The incidence was 13.3% among males and 16.3% among females, with no significant difference (p-value=0.786). There was significant association between ANA and lupus in both genders. Only one patient with negative ANA and 81.8% of patients with positive ANA developed lupus (P<0.005).

Summary/Conclusions: SLE developed in patients with primary ITP with initial positive ANA titer at presentation. The results suggest that patients with initial positive ANA are at risk for development SLE. Thus, follow up after primary ITP diagnosis with positive ANA titer is of great importance as the risk of SLE is significant.

PB2122

TREATMENT OF REFRACTORY IMMUNE THROMBOCYTOPENIA WITH THROMBOPOIETIN RECEPTOR AGONISTS: OUR EXPERIENCE IN CHILDHOOD

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Background: Immune thrombocytopenia (ITP) is an autoimmune disease in which antibodies develop against platelets (plts) and dysregulation of cellular immunity result in premature destruction of plts and impaired platelet production. For most affected children, ITP is a self-limiting disease. Approximately, 10% of all ITP patients eventually develop refractory ITP (RITP). Thrombopoietin receptor agonists (TPO-RA) stimulate thrombopoiesis and are an alternative to Rituximab (Rtx) for patients with positive ANA developed lupus (P<0.005).

Aims: We present 3 different children with RITP treated with TPO-RA.

Methods: CASE 1. A 5-year-old girl admitted to the hospital due to ITP with mucocutaneous bleeding. She was refractory to corticoids, immune globulin (Ig) and anti-D Ig. Rtx was started. After the 3rd dose, she responded temporaril ory along with fever, renal insufficiency and arterial hypertension, probably related to Ig A deficiency, not previously diagnosed. Romiplostim was indicated, reaching complete remission after 2 doses and it was stopped after the 4th dose, without any adverse reaction. Nowadays, plt count remains within normal limits (Figure 1A). CASE 2. A 5-year-old boy was diagnosed of ITP with cutaneous bleeding. He received treatment with prednison and Ig with short response. Rtx was indicated; after 4 doses, thrombocytopenia and cutaneous bleeding persisted. Eltrombopag was started with response after 6 weeks of treatment (Figure 1B) and bleeding symptoms recovery. CASE 3. A 4-years-old boy with RITP was referred to our hospital. We decided to initiate treatment with Eltrombopag. He developed response after 4 weeks of treatment reaching complete remission after 2 doses of 75mg/24h. Six weeks later, he presented 600,000/plts/µL, so the drug was stopped. We observed a quick descent in plts levels and Eltrombopag was restarted with progressive response (Figure 1C).

Results: In all cases, splenectomy was avoided due to long-term risk of sep, as well as immunosuppressive agents like RTX in 3rd case. In 1st case, TPO-RA was able to stop with sustained response as described in some publications.

Summary/Conclusions: In our experience, TPO-RA appear to be efficacy and well tolerated in children.

PB2123

INVESTIGATION OF PLATELET FUNCTIONS IN PSEUDOTHROMBOCYTOPENIA

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Background: Pseudothrombocytopenia (pseudoTCP), 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, pseudoTCP 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 in theory, formation of platelet clusters in the presence of EDTA requires functional adhesion molecules, so platelet adhesion and aggregation tests are expected to be in normal range. We aimed to investigate the capacity of simple platelet function analysers for making the distinction between pseudo TCP and real thrombocytopenia.

Methods: Platelet functions were measured as collagen-ADP and collagen-epinephrine closure times (ColADP and ColEPI) by Platelet Function Analyzer (PFA-200™) for all patients who were referred to our clinic as thrombocytopenia (TCP, plt<150 x10^9/µL) and 11 healthy controls (Plt>150 x10^9/µL). Median age was 54 (IQR:37-68) for thrombocytopenic subjects and 37 (%63) of them were female. Median Plt count was 61 x10^9/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 pseudoTCP and real-TCP. There was no difference in terms of Plt, MPV, PCT, WBC or Hb between these groups but age was younger (median age 46 vs 62, p<0.05) and PDW was higher in pseudoTCP group (med 17.6 vs 16.8, p<0.01). ColEPI and ColADP measures were significantly lower (med 125 vs 287 for ColEPI, med 84 vs 224 for ColADP, p<0.001 for both) at pseudoTCP group. The capacity of ColEPI and ColADP values in predicting pseudoTCP 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: 0.694-0.933). 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 with thrombocytopenic counts, could be used for differentiate pseudoTCP and realTCP in centers which does not have conditions for proper BS. Especially long closure times excludes pseudoTCP with a high specificity and could make clinicians quick decisions for further investigations.

Figure 1.
BACKGROUND: The investigation and management of patients with Chronic immune thrombocytopenic purpura (ITP) varies widely. Although many treatments have been recommended for ITP, there are no evidence-based recommendations for when different treatments should be used, or when any treatment should be used rather than managing a patient by observation alone.

AIMS: To evaluate the treatment of ITP patients in Department of Hematology, County Hospital, Timisoara.

METHODS: A retrospective study for 350 ITP patients was performed. Patients demographics, medical history, current treatments and side effects, were abstracted from the patient’s medical charts for the 15 months prior to their most recent visit.

RESULTS: The mean age was 45.6 years with 58% women and 42% men. Median time from the diagnosis of ITP to the start of the observational period was 23 months. Regardless of the presence of bleeding symptoms, for majority of patients we started treatment based on plateled 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 IVlg (20%), azathoprine (12%) rituximab and 8% others. Splenectomies (8% of patients) and platelet transfusions (27% of patients) were performed during the observational period. In the patient survey, 52% of participants were 60 years of age or older and the duration of disease was more than 10 years in 43% of patients. The minimum platelet counts were less than 10x10^9/L in 49% of patients. The most common symptoms of ITP was fatigue (45%). Approximately 60% of patients reported at least one side effect associated with ITP treatment. The side effects were most frequently associated with corticosteroid use (43%). Overall, 40% of patients required hospitalization. Mean duration of hospitalization was 13.5 days.

SUMMARY/CONCLUSIONS: The retrospective study of 350 patients provides the results of treatment practices in our country. It showed that bleeding symptoms remained quite frequent among patients with chronic ITP. Corticosteroids were the most widely used treatment.

PB2125

IMMUNOLOGICAL THROMBOCYTOPENIC PURPURA AND PREGNANCY: A RETROSPECTIVE STUDY OF 89 PREGNANCIES IN 59 PATIENTS

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BACKGROUND: Immunological thrombocytopenic purpura (ITP) occurs for about 1 case for 1000 pregnancies. The risk of onset, aggravation or relapse of ITP during pregnancy is not clearly established.

AIMS: The aim is to describe the prevailing ITP progression profile in pregnant women and to evaluate the risk of neonatal thrombocytopenia in two situations, when ITP was known before pregnancy and when ITP was discovered for first time during pregnancy.

METHODS: It is a retrospective study carried out in the hematology department of CAC Blida, Algeria, between 1993 and 2016. All patients (pts) who had a pre-pregnancy ITP or thrombocytopenia during pregnancy attached to an ITP were included.

RESULTS: The development of 89 pregnancies (PG), including two twins, occurred in 59 women was analyzed. There were one PG in 40 pts, 2 PG: 13 pts, 3 PG: 5 cases, 4 PG: 1 case and 5 PG: 1 case. Of the 59 pts: in 42 cases it was a history of ITP before pregnancy (group 1: G1) with a history of splenectomy in 9 patients, and in 17 cases it was ITP discovered on the occasion of Pregnancy (group 2: G2). The average age at diagnosis=26.7 years (7-44) and that at delivery=30.4 years (19-44). The mean platelet count at diagnosis: G1: 34000 / µL , G2: 47000 / µL. 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, abstinence 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=12), 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, and 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=1) transfusion of platelets (n=4), immunoglobulins in 4 cases and transfusion of platelets alone in 4 cases. At birth, thrombocytopenia was observed in 40 pregnancies (50.6%): platelets <30000 / µL (n=7), between 31000 and 50000 /µL (n=13), between 51000 and 100000/µL (n=20), between 100000 and 150000/µL in 2 cases. All pregnancies were completed: 14 by caesarean section, one for thrombocytopenia, with an average platelet count=95000 /µL and 75 by natural delivery with a mean platelet count=100000 / µL with 4 deaths born, one anencephaly and 88 newborns. No hemorrhagic syndrome was observed in pregnancy; two postpartum hemorrhages were seen in G2 group. Eleven newborns (5 in G1 and 6 in G2) were thrombocytopenic with platelet count <20000/µL in 4 cases; between 20000 and 50000/µL in 7 cases; neonatal thrombocytopenia occurred during the first 7 days. Only 4 newborns were treated, one by corticosteroid and 3 by immunoglobulins, with a good progression and only one of the untreated is always followed for thrombocytopenia.

SUMMARY/CONCLUSIONS: The de novo ITP appearing during pregnancy is an etiological eventuality to be evoked in front of a thrombocytopenia of the pregnant woman after elimination of the other causes related to the pregnancy and in front of the non-resolution after the delivery. The pre-existing ITP does not necessarily.
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), hematologic neoplasia of other st. (n=126), Non-Hodgkin's lymphoma in III-IV st. (n=40) and chronic lymphocytic leukemia in B or C st. (n=67). Patients were examined: 1) clinical blood test (hemoglobin concentration) to assess anemia’s grade; 2) the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale to measure of QoL. The FACT-An questionnaire consists of a general questionnaire (FACT-G), measuring domains of physical well-being (PW), social/family well-being (S/FW), emotional well-being (EW), functional well-being (FW), an 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 (Me=5.7 g/dl); 2) the second – Hb 6.5-7.9 g/dl (Me=7.2 g/dl); the third – Hb 8.0-9.4 g/dl (Me=8.6 g/dl); the forth – Hb 9.5-10.9 g/dl (Me=10.8 g/dl); the fifth – Hb 11.0-11.9 g/dl (Me=11.4 g/dl); the sixth – Hb 12.0-14.4 g/dl (Me=13.0 g/dl). In the sixth group of patients (n=41) with anemia grade 0 QoL in PW was 11.0±1.0, in S/FW – 14.9±0.8, EW – 7.6±0.6, FW – 16.4±0.5, AnS – 34.6±2.2, FS – 23.7±1.6, NFS – 10.9±1.7. In the sixth group of patients (n=56) without anemia QoL in the subscale of PW was 13.4±0.6, in S/FW – 13.6±0.5, in EW – 6.4±0.5, in FW – 14.8±0.7, AnS – 23.4±1.5, FS – 14.9±1.0, NFS – 8.4±0.6.

Summary/Conclusions: QoL was found too poor in patients with Hb <8.0 g/dl. QoL wasn’t satisfactory in patients with Hb 8.0-11.0 g/dl. But the QoL improvement were greater in patients with Hb levels >11.0-12.0 g/dl (p<0.05). These data suggest that early correct anemia with red blood sells transfusions and erythropoiesis-stimulating agents can improve QoL in a clinically meaningful way.

PB2128
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 LMWH (prefilled treatment syringes 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 Canada 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 was 19% of the total cost of the 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.

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Madrid, Spain, June 22 – 25, 2017
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 chemo 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 Gel X® in chemotherapy induced mucositis. GelX® is a topical product that contains Zinc gluconate-taurine, with bactero-bactericidal and anti-inflammatory effect, easy to use for the patient, in order to prevent and reduce pain and severity of oral ulcers, making a barrier for mucositis.

Methods: A retrospective analysis of 77 adult patients: 17 with hematological treatments and 60 with allologenic stem cell transplantation. 17 were diagnosed and treated between January 2015 and December 2016 with various hematological malignancies (5 AML, 2 ALL – 1 Ph positive, 2 blastic phases of CML, 3 BMF, 1 BH), 16 cases of grade 3-4 mucositis. The regimens used were 6 mieloablative and 15 nonmieloablative. Tumors included: ALL, 1 ATLL, 5 LMNH, 1CLL, 2 SAA, 2 CML, 1 mycosis) with 10 cases of mucositis grade 3-4. The regimens used were 6 mieloablative and 15 nonmieloablative.

Results: Prophylaxis induced a reduction in the grading of mucositis (time to heal shorter than 10 days). Identifying candidates for mucositis prophylaxis. In 60 patients allografted for various hematological conditions (35 AML, 4 ALL, 2 SA, 2TLL, 2 M MM, 2 CML, 1 MDS, 1 BH), 16 cases of grade 3-4 mucositis has appeared. The conditioning regimen was mieloablative (14 cases) and reduced intensity (21 cases). There were 21 cases of sibling allotransplants (GAML, 3 ALL, 1ATLL, 5 LMNH, 1 CML, 2 SAA, 2 CML, 1 mycosis) with 10 cases of mucositis grade 3-4. The regimens used were 6 mieloablative and 15 nonmieloablative. 3 from 4 cases of haplotransplant with nonmieloablative conditioning (2MDS, 1 AML and 1 SAA) had grade 3 mucositis.

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: Almost all hematological disorders are rare diseases, affecting less than 1 in 2000 individuals, justifying their inclusion in a European Reference Network (ERN). ERN are networks created following the Directive 2011/24/EU on cross border health care. They are supported by a rare disease section of the European Reference Network on Rare and Congenital Anaemias (ENERCA), the European Haematology Association (EHA), and European hematologist patient organisations representing in both the EURODID 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.

Objectives: EuroBloodNet’s main role 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 and complex diseases 4) Providing access to clinical trials and registries and safe exchange of clinical information 5) Fostering European cooperation in highly specialized procedures for diagnosis, promotion of clinical trials and innovative treatments and research.

Methods: RHD are covered in two main thematic groups: non-malignant diseases 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, improve guidelines and best practice, to improve training and knowledge sharing, to offer clinical advice where national expertise is scarce, and to increase the number of clinical trials in the field.
separately analyzed only those with young stroke (Age <45). In this cohort there were 420 patients. A total of 6 (1.4%) patients had potential PV based on the 2008 Hb criteria. On applying the 2016 revision; 37 (8.8%) patients fulfilled the Hb criteria. An exact McNemar’s test determined that there was a statistically significant difference in the proportion of polycythemias, p=0.000. Separate analyses by gender was not significant in females, χ²=0.5; but significant in males, χ²=10.91, p=0.001. Indian males with the revised criteria for polycythemia. The impact of cost in influencing treatment decision from resource limited countries with predominant out of pocket health expenditure has been earlier reported (Philip C et al, 2015). This revision promotes the routine use of BM and JA-2. In our analysis we estimate this new criteria would add to the cost to each patient (~7000 per our centre estimate).

**Summary/Conclusions:** The present data shows that there exists a significant difference in the incidence of polycythemia in thrombosis (Ischaemic Stroke) on applying the revised criteria. The requirement to additionally investigate them with BM and molecular markers for PV has potential economic implications.

**PB2132**

**PATHOPHYSIOLOGICAL MECHANISMS INVOLVED IN THE DEVELOPMENT OF ANEMIA IN PATIENTS WITH NON-HODGKIN’S LYMPHOMA**

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**Background:** Non-Hodgkin’s lymphomas (NHL) are a group of heterogeneous malignant lymphoid disorders that associate anemia either from diagnosis or during the evolution of the disease. The anemic syndrome can be present at the moment of diagnosis or can develop during the evolution of non-Hodgkin’s lymphomas, with or without a hematological remission due to the action of intensive and density of drug doses, overall survival and quality of life of these patients. 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 in this study group.

**Methods:** A retrospective study was conducted on 85 patients (informed consent obtained) with non-Hodgkin’s lymphoma, who were admitted to the Clinics of Hematology, Filantropia City Hospital, Craiova, Romania, in between 2013 and 2015, in order to evaluate the prevalence and pathophysiological mechanisms involved in the development of anemia in this study group.

**Results:** In our study group, the median age at diagnosis of non-Hodgkin’s lymphoma was 64 years, sex distribution was males:females=1:3, and the rural to urban area index=1:2. 85.88% of patients had B type NHL and 14.12% T type NHL. 20% of NHL were indolent lymphomas, aggressive lymphomas in 54% cases. NHL, according to Rai staging, revealed: type I – 2.35%, type II – 18.81%, type III – 57.64%, and type IV – 21.16%. In our study group, 84% of patients enrolled had anemia, with the anemic syndrome affecting the 50-59 years and 70-79 years age groups. 59.73% of patients had anemia at diagnosis and 40.27% of patients developed anemia during the evolution of NHL. The pathophysiological mechanisms involved in the development of anemia were: perturbations of iron metabolism and erythropoiesis under pro-inflammatory cytokines and hepcidin 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 cause was present in 9.7% of cases. In all cases, they had at least one manifestation that prolonged hospitalization, endangered life or was death-associated. Most frequent AE reported was acute reaction related to chemotherapy in 9 cases (1 cardiac failure and 1 ischemic stroke), infections (1 pneumonia, 1 progressive multifocal leukoencephalopathy), neurologic (1 paresthesia), cytopenias (1 pancytopenia) and cutaneous (1 bullous dermatitis).

**PB2134**

**DEPRESSION AS THE PRESENTING SYMPTOM OF CENTRAL NERVOUS SYSTEM LYMPHOMAS IN NORTHWESTERN TURKEY**

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**Background:** PCNSL represents approximately 4 percent of newly diagnosed primary central nervous system (CNS) tumors, with an age-adjusted incidence rate of four cases per million persons per year. Most cases of non-AIDS related PCNSL are diagnosed in patients between 45 and 65 years of age, with a median age at diagnosis in the fifth decade. The most notable risk factor for the development of PCNSL is immunodeficiency including HIV infection, iatrogenic immune suppression, and congenital immune deficiencies. Antecedent flu-like or other upper respiratory illnesses or a history of autoimmune diseases were reported. Presenting symptoms may include focal neurologic deficits, neuropsychiatric symptoms, signs of increased intracranial pressure, seizures or ocular symptoms. Neuropsychiatric symptoms like depression, anxiety, psychosis, confusion, memory impairment, slowness of thought are generally underdiagnosed or attributed to the treatment-related effects of depression, cancer, and the antidepressant use. Diagnosis is based on imaging of the central nervous system (CNS), ideally with contrast-enhanced magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, unless contraindicated due to elevated intracranial pressure. The radiographic lesion tends to be a solitary non-hemorrhagic mass, situated in the deep white matter adjacent to the ventricular surface.

**Aims:** We aimed to evaluate the presence of depression and antidepressant use before the diagnosis of CNS lymphoma and emphasize the duration between the diagnosis of depression and lymphoma.

**Methods:** Data of 40 patients with CNS lymphoma were evaluated in a retrospective manner. From their national health records, prescription for antidepressant and anxiolytic drugs with their psychiatric diagnosis, time before the diagnosis of CNS lymphoma, the branch of the prescribing physician, presenting symptoms

**PB2135**

**SAFETY OF RITUXIMAB BIOSIMILAR (NOVEX®) IN THE ROUTINE USE TREATMENT IN ARGENTINA.**

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**Background:** Novex® is a biosimilar by design of the reference product Mabthera®/Rituxan®. Novex® was approved in Argentina following ANMAT’s Biosimilar guidelines, having the same indications as the reference product, Rituxan® is commercialized by Laboratorio Elea. As part of the Risk Management Plan (RMP), Laboratorio Elea implements an active pharmacovigilance program as defined in Argentina regulation. Periodically reports ANMAT RMP status and results.

**Aims:** To describe frequency and pattern of adverse events during the use of Novex® in treatments registered along active pharmacovigilance program in order to oversee the safety profile of NOVEX® in the real clinical practice and maintain the benefit-risk evaluation.

**Methods:** A treatment Registry for NOVEX® was implemented from the beginning of NOVEX® commercialization as part of the RMP. The Data Lock Point for this report is Jan 31st, 2017. Physicians prescribing NOVEX® were request-
ed to fill a form indicating age and gender, treatment start date, treated pathol-
ology, dosage and dose frequency. Such data was recorded in a database. After a preset time, physicians were contacted by Laboratorio Elea to ask them about the treatment outcome and Adverse Event occurrences. If adverse events were detected they reported each occurrence as defined in the RMP. Specifically, cases with severe adverse reactions and/or death (ICSR) were registered using the MedDRA dictionary (version 19.1) for its codification.

**Results:** The total number of participating physicians was 151. During this period, they reported 638 treatment intitutions, 389 of which had at least 1 follow up point and 414 were included in further analyses. 53% male, mean age 64.1 years. Patho-

**Summary/Conclusions:** The activities developed under this active pharma-
covigilance program showed great value allowing us not only to monitor the adverse event pattern but also to detect off-label use as part of real life treatments. This report showed a similar safety profile to that of the reference product concluding that NOVEX®, in terms of tolerability, is similar to the reference product. Pharmacovigilance is cornerstone in the development of biologics, especially biosimilars, as a tool to assist in the knowledge about their safety profile.
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 male (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 neuropsychiatric 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 the diagnosis was classified 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 common under diseases 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 TRANSPLANT ON TOTAL HEALTHCARE COSTS
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Background: Lenalidomide maintenance therapy after autologous hematopoietic stem cell transplant (auto-HSCT) in the first-line treatment has been shown to improve progression-free survival (PFS) and overall survival (OS) in multiple myeloma (MM) patients.

Aims: This study assessed the budget impact of the United States (U.S.) Food and Drug Administration (FDA) approval of lenalidomide maintenance therapy on total healthcare costs of a U.S. health plan.

Methods: An economic model was developed to estimate the incremental (additional) total plan costs (in 2016 USD) of maintenance therapy in each year for the first 3 years after lenalidomide monotherapy (R) maintenance therapy approval. The number of post auto-HSCT adult MM pts eligible for initiating maintenance therapy was estimated from published epidemiological data and an analysis of Connect® MM Registry data. Clinical endpoints for R-maintenance, including time on treatment, PFS and OS, were obtained from a meta-analysis of published clinical trials (CALGB, IFM, and GIMEMA). The use of common off-label maintenance therapies was considered. Types of costs included in the model were drug, drug administration, adverse events (AE), AE monitoring, one-time progression and terminal care costs.

Results: In a hypothetical U.S. health plan with 1 million members, the number of adult MM pts eligible to initiate post-auto-SCT maintenance therapy was estimated to be 28. Among them, 14.8 pts initiated R-maintenance in Year 1, 15.2 in Year 2, and 15.3 in Year 3, representing an incremental increase of 2.9%, 4.2% and 4.4% after R-maintenance therapy approval, respectively. After considering additional costs of maintenance, as well as potential offsets resulting from delayed progression the incremental total healthcare costs by year are listed in the Table 1. Results were consistent across all total plan, per patient, per year, and per member per month costs. Deterministic sensitivity analysis showed that the model results were robust to the variations of key model inputs.

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 HEMOPATIES
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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 cytopoenia. 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 hematological malignancies is still unclear. Nevertheless, the ageing of the world’s population and the increased incidence of Non-Hodgkin’s Lymphoma are increasing the indications for splenectomy, requiring a well-tolerated and less invasive procedure.

Aims: The aim of this review is to analyze our single-center experience of LS performed for malignant Hemopathies. Results are compared with LS for benign splenomegaly and the risk of locoregional dissemination or inadequacy of fragmented histological sample were analyzed.

Methods: We retrospectively analyzed 50 patients who underwent LS between 2005 and 2016 at Saint-Pierre Hospital. Among which 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 following chronic lymphocytic leukemia (7), hairy cell leukemia (1) and Hodgkin’s lymphoma (1). Patients’ age (67 +/- 12 years, ranging from 36 to 87 in SMH, 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 (76% vs 71%) and SMH and SBH group. Out of 31 patients received a pre-surgical corticosteroid treatment, with a pre-surgical platelets level of 156 +/- 108 x 103/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, LSe 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? M.H. Dogu1, 2, R. Eren1, N. Nizam2, O. Yokus1, E. Suyan1.
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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 patients' mental health, that is why patients need great attention to be supported. Depression and anxiety are the most common psychological complaints in patients with acute leukemia. Therefore, it is important to have awareness of these psychological problems in patients with acute leukemia.
Background: Three years ago, a unit for autologous bone marrow transplant for hematological patients has been established in Shaare Zedek medical center. The patients meet with the doctors for the treatment plan usually following the diagnosis. From the point of view of a part of the patients, the process appears simple, short term, and promises cure. In reality, the process is long term, including aggressive chemotherapy prior to the transplant. The treatment is highly aggressive and toxic with many physical and mental side effects for the patient and his/her family. The transplant process requires hospital admission for about a month in an isolation room. No one is allowed in the room except for close relatives and the medical staff. The social worker, part of the caring staff, accompanies patients and families from the initial diagnosis through this taxing and stressful process. Most patients are young, average 45 years, in the middle of their careers, from a broad spectrum of occupations, education as well as social status, representing Israeli society.

Aims: To accompany and empower patients by means of giving them tools to cope with the transplantation process which is a crisis situation in the midst of their lives. To teach patients self-awareness. 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 for the patients during the transplantation process is the coping with it. The process of treatment helps patients to go from the private sphere back to the public one.

Recommendations: It seems essential for the patients in the isolation room, undergoing autologous bone marrow transplant, to have therapy sessions with a qualified social worker as part of the holistic care. ‘Having a room of his own’ in the process enables an opportunity to examine the inner self esteem and strengths of the patients thereby patients learn to contribute to themselves from themselves.

PB2138

GENDER DIFFERENCE IN ANXIETY FOR THE FIRST BLOOD TRANSFUSION

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Background: Blood transfusion has several risks including allergic reaction, acute hemolysis, infectious disease and so on. Both physicians and patients are always cautious to decide on blood transfusion.

Aims: The purpose of this study was to explore whether there are gender differences in anxiety for the first blood transfusion in patients with different diseases.

Methods: 315 patients (153 men and 162 women) were enrolled in this prospective, comparative study and median age was 38 years(range 17-72). The disease consisted of 85 chronic hepatitis B, 73 leukemia, 69 gastric ulcer, 48 chronic renal failure and 40 gynecological oncology. Various blood products including plasma, red blood cells suspension and platelet were infused. Anxiety was evaluated according to the 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 Aydemir et al (9). The questionnaire has a total of 14 items: seven of which measure anxiety (odd numbers) and the remaining seven (even numbers) measure depression. Each item is scored from 0 to 3. The scoring order of each item in the questionnaire is different. Items numbered 1, 3, 5, 6, 8, 10, 11 and 13 indicate decreasing severity and are scored as 3-2-1-0. On the other hand; items numbered 2, 4, 7, 9, 12 and 14 indicate increasing severity and are scored as 0-1-2-3. The cut-off value for the total score of the odd-numbered questions assessing anxiety is 10; while it is 7 for the even-numbered questions assessing depression.

Results: 21 patients were included in the study. 13 of these patients (61.9%) were diagnosed with acute myeloid leukemia (AML) and 8 (38.1%) were diagnosed with acute lymphoblastic leukemia (ALL). Median age of the patients was 45 (range: 21-69). 11 patients (52.4%) were female and 10 (47.6%) were male. 5 patients (23.8%) had comorbidities while 16 (76.2%) had none. Anxiety evaluation revealed that 38.1% of all patients in the study experienced anxiety. The rate of anxiety was 38.5% in AML patients and similarly 37.5% in ALL patients. 45.5% of the female patients had anxiety while the rate was only 30% in male patients. The difference was not statistically significant (p >0.05). Depression evaluation revealed that 81% of all patients in the study. The rate of depression was 84.6% in AML patients and 75% in ALL patients. 81.8% of the female patients had depression while it was 80% in male patients. Neither anxiety nor depression had a significant correlation with comorbidities or gender (p >0.05). Correlation analysis revealed a positive correlation between anxiety and depression (r=0.846; p <0.01).

Summary/Conclusions: In conclusion, assessing anxiety and depression in patients with the same disease, more female patients were divided into severe anxiety group than male ones. The number of patients with mild anxiety was similar in female and male, and no one was divided into no anxiety group.

PB2139

A ROOM OF MY OWN

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Summary/Conclusions: Women were more anxious than men during the first blood transfusion, which is independent of age, race, education level and kinds of blood product.
**Sickle cell disease**

**PB2140**

**HYDROXYUREA INHIBITS MYELOID DIFFERENTIATION VIA NITRIC OXIDE SYNTHASE**

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**Background:** Hydroxyurea and nitric oxide (NO) inhibit erythroid differentiation, while hydroxyurea is NO-releasing agent used in therapy of sickle cell diseases in order to decrease oxyhemoglobin levels and prevent vaso-occlusions. Therefore, NO metabolites might have a neurodevelopmental impact if left untreated.

**Aims:** To study the mechanism of hydroxyurea inhibition of erythroid differentiation by exploring NO synthase (NOS) dependence.

**Methods:** The erythroid differentiation is studied by methylcellulose colony assay in mice, whereas presence and activation of endothelial NOS (eNOS) by immunocytochemistry and immunoblotting, respectively in K562 erythroleukemic cell line.

**Results:** In ex vivo experiments, mice exposed 7 days to hydroxyurea demonstrated significant decrease in the number of nucleated cells per femur, partially reversed by NOS inhibitor N-nitro-L-arginine methyl ester hydrochloride (L-NAME). The same, but less prominent reduction has been observed with NO metabolites nitrite (NO2) and nitrate (NO3). Moreover, hydroxyurea demonstrated a large diminution in the number of bone marrow derived myeloid colony-forming unit-granulocyte/macrophage (CFU-GM), burst-forming-units-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) colonies in methylcellulose cultures. L-NAME attenuated hydroxyurea reduction of myeloid and erythroid colonies, while by itself increased CFU-E and CFU-GM colonies and slightly BFU-E colonies. NO metabolites NO2 and NO3 generally inhibited myeloid and erythroid colonies, but the reduction was more prominent by NO2 compound. Moreover, the hematological parameters and weight (before and after treatment) of mice did not show any significant difference among studied groups. Hydroxyurea increased NO production and the number of eNOS positive K562 erythroleukemic cells, while phosphorylation of eNOS and activation of AKT/mTOR signaling was not blocked by phosphatidylinositol 3-kinase inhibition.

**Summary/Conclusions:** NO prodrug hydroxyurea demonstrated NOS dependence in inhibition of myeloid / erythroid differentiation, not influencing the hematological parameters.

**PB2141**

**SLEEP DISORDERED BREATHING IN CHILDREN AND ADOLESCENT WITH SICKLE CELL DISEASE: IMPACT ON EXECUTIVE FUNCTION AND PROCESSING SPEED INDEX**

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**Methods:** To study the mechanism of hydroxyurea inhibition of erythroid differentiation by exploring NO synthase (NOS) dependence.

**Results:** A total of 101 children and adolescents were included (n. 62 in UK; n. 39 in Italy; 42% girls; age-range: 6-21.7 years). We didn’t find significant differences in mean spirometry indices between the SCA cohort from London and northeast Italy (Table 1). Nevertheless while an obstructive spirometric pattern was more common in the British cohort compared to the Italian one (respectively 22.5% vs 7.7%), the picture was the opposite for the restrictive pattern (respectively 11.2% and 20.5%) (Table 1). In the whole sample age was negatively correlated with both zFEV1 (Spearman’s rho -0.20) and zFVC (Spearman’s rho -0.24).

**Table 1.**

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<tr>
<th>Variable</th>
<th>Sickle cell UK</th>
<th>Sickle cell FTA</th>
<th>Diff between mean (5%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>11.9 (6.2)</td>
<td>11.3 (6.5)</td>
<td>0.64 (0.2 to 1.0)</td>
</tr>
<tr>
<td>Height (z-score)</td>
<td>-0.11 (0.23)</td>
<td>-0.28 (0.99)</td>
<td>-0.15 (0.2 to 0.6)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.5 (3.5)</td>
<td>18.5 (3.0)</td>
<td>0.02 (0.5 to 0.9)</td>
</tr>
<tr>
<td>FEV1 percentage</td>
<td>78.0 (12.7)</td>
<td>78.0 (12.7)</td>
<td>0.00 (0.0 to 0.0)</td>
</tr>
<tr>
<td>FEV1/FVC ratio</td>
<td>0.67 (0.16)</td>
<td>0.67 (0.16)</td>
<td>0.00 (0.0 to 0.0)</td>
</tr>
<tr>
<td>Spirometry</td>
<td>0.62 (0.19)</td>
<td>0.62 (0.19)</td>
<td>0.00 (0.0 to 0.0)</td>
</tr>
<tr>
<td>Z-scores</td>
<td>0.62 (0.19)</td>
<td>0.62 (0.19)</td>
<td>0.00 (0.0 to 0.0)</td>
</tr>
<tr>
<td>Normative parameters</td>
<td>0.62 (0.19)</td>
<td>0.62 (0.19)</td>
<td>0.00 (0.0 to 0.0)</td>
</tr>
</tbody>
</table>
| Summary/Conclusions:** NO produg hydroxyurea demonstrated NOS dependence in inhibition of myeloid / erythroid differentiation, not influencing the hematological parameters.

**Summary/Conclusions:** Lung function of pediatric subjects with SCA living in London and in the northeast of Italy is overall comparable. Obstructive lung disease is more common among subjects with SCA living in London than in urban and non-urban areas in Italy. Differences in the level of exposure to ambient air pollution and in the prevalence of allergies between the rural and urban environment might have contributed to this finding and need to be further investigated.

**PB2142**

**LUNG FUNCTION IN CHILDREN AND ADOLESCENTS WITH SICKLE CELL ANEMIA: A COMPARISON BETWEEN UK AND ITALY**

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**Background:** Acute and chronic respiratory complications are common in sickle cell anemia (SCA). Subjects with SCA often have a progressive decline of lung function with age that could be influenced by the quality of healthcare and by environmental factors, such as the level of exposure to air pollution.

**Aims:** To compare lung function, evaluated cross-sectionally through spirometry, in children and adolescents attending sickle cell centers in UK and Italy.

**Methods:** Anthropometry and spirometry were recorded in patients with SCA (SS,SCD) aged 6-17 years of African ancestry followed at the Evelina Children’s Hospital, London, UK, and at the University Hospitals of Padova and Udine, northeast of Italy. Subjects from the British cohort lived in an urban area while those from Italy came from urban and non-urban areas. Exclusion criteria were the presence of SCA-related morbidity within the last two weeks and the inability to perform a spirometry meeting the European Respiratory Society acceptability and repeatability criteria (Miller, Eur Respir J 2005;26:319–338), modified for children (Kirkby, Pediatr Pulmonol 2008;43:1233–1241). Portable spirometers (Pony FX, Cosmed-IT, Easy-on PC, NDD-CH) were used. Z-scores of anthropometric and spirometric data were derived, respectively, from CDC2000 and from the Global Lung Initiative 2012 predictive equations for African Americans (Quanjer, Eur Respir J 2012: 40:1324–1343). Spirometry patterns were classified as normal, obstructive (zFEV1/zFVC<1.64) or restrictive (zFVC<1.64+zFEV1/zFVC ≥ -1.64). Differences between groups were assessed by t-tests and considered statistically significant for p values <0.05.

**Results:** A total of 101 children and adolescents were included (n. 62 in UK; n. 39 in Italy; 42% girls; age-range: 6-21.79 years). We didn’t find significant differences in mean spirometry indices between the SCA cohort from London and northeast Italy (Table 1). Nevertheless while an obstructive spirometric pattern was more common in the British cohort compared to the Italian one (respectively 22.5% vs 7.7%), the picture was the opposite for the restrictive pattern (respectively 11.2% and 20.5%) (Table 1). In the whole sample age was negatively correlated with both zFEV1 (Spearman’s rho -0.20) and zFVC (Spearman’s rho -0.24).

**Summary/Conclusions:** Lung function of pediatric subjects with SCA living in London and in the northeast of Italy is overall comparable. Obstructive lung disease is more common among subjects with SCA living in London than in urban and non-urban areas in Italy. Differences in the level of exposure to ambient air pollution and in the prevalence of allergies between the rural and urban environment might have contributed to this finding and need to be further investigated.
Methods: The study is observational, uncenteric, descriptive and retrospective, carried out in February 2017 in a tertiary hospital in the Community of Madrid, Spain. All patients diagnosed with SCD and who had attended at least once to the hematology clinic for this reason were included. Demographic characteristics (date of birth, gender, country of birth) and clinical characteristics (genotype, therapy and update in follow up, like alive, deceased or lost patient) were collected from the patient’s file. Written consent was signed by patients or legal guardians in accordance with the Declaration of Helsinki. The study was approved by the hospital Ethical Committee. Statistical analyses were performed using SPSS version 18.0. Quantitative variables were reported as median or mean value and range, while categorical variables were expressed as absolute value and percentage.

Results: The total number of SCD patients included was 209. Ratio boy/girl is 1.3. Most of patients were born in Spain (85%), although 8% and 5.26% were born in Africa or America respectively. Seventy three percent of the progenitors came from Africa and 24% from America. Ninety two percent of those SCD patients born in Spain were detected in the first days of life due to universal screening detection implemented in Community of Madrid since 2003. Median age at first diagnosis was 1.42 years (0-21.4). Median age at the end of inclusion was 9.91 years (range 0.13 to 35.14). SS or S/Betathal was reported in 86%. In addition, 2.39% associated alla gen deletion, and in 1 (0.48%) glucose 6 phosphate dehydrogenase deficiency. No patient had congenital thalassemia or diabetes. Eighteen patients (8.65%) had human leucocyte antigen (HLA) identical siblings. Hydroxyurea was added to standard treatment in 65 patients (31%) of which 47 continue to be treated to date. Penicillin prophylaxis was communicated in 165 patients (79%). Vitamin-D prophylaxis was initiated in 122 patients (60%). Blood transfusion exchange or splenectomy transusions existed in 25 cases (12%) and 9 children (4%) underwent splenectomy. None of these patients had sepsis or meningitis. Cholecystectomy was performed in 9 cases (4%). There were 18 progenitor stem cell transplantations (8.61%) performed between 2.09 to 13.87 years of age (median 6.77 years). Ten patients remained on eculizumab therapy at the end of the study (p=0.15). Eighteen patients (8.65%) had a history of painful crisis. Fourteen patients (6.72%) had significantly increased HbS levels (80.5±11). None of these patients had organ dysfunction. No significant difference was found regarding age, gender, platelets, white blood cells, Hb, HfB, 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. Effective inhibition of complement activation in vitro is promising for future studies in selected patients.

Background: Childhood with Sickle Cell Disease (SCD) frequently present impairment of general and specific neurocognitive functions, even in the absence of clear neurological damage at brain neuroimaging. Verbal language deficits are also common, but the etiology of poor performance in the verbal domain is still not clear. The ability to speak and communicate verbally relies on a complex interaction of cognitive and linguistic functions as well as on environmental factors, like bilingualism or second language (L2) learning. The majority of children with SCD in Italy are of immigrant families whose first language is not Italian. These children thus perform poorly in tests assessing the verbal domain with adverse impact on school performances.

Aims: To evaluate if verbal language deficits in bilingual children with SCD are mainly due to linguistic and environmental issues or to impairment of executive functions.

Methods: In this study a cohort of bilingual children with SCD and social-demographically matched healthy controls recruited from elementary schools of the same school, performed an extensive battery of tests to assess naming skills, phonological and semantic fluency, attention and Executive Functions (inhibition and planning skills) and visuo-spatial skills (Boston naming test, phonological and semantic fluency tests, Flanker task, Eilthorn test, PMA spatial relations subscale). All tests were administered in Italian. A composite index considering parental education and employment was used to match socially, demographically and economically children at best. Analyses of variance (ANOVAs) were performed to test differences between the two groups in verbal language, attention and executive functions. Hierarchical regressions explored the contribution of linguistic knowledge and executive functions (i.e. inhibition) to the verbal language deficit of children with SCD.

Results: Thirty two children with HbSS SCD aged 6 to 12 years (mean age=9.03) and 35 controls (mean age=9.14) were enrolled. Patients and controls were matched for gender (F 53 vs 61%, ethnicity (African 30 vs 29%), % of children born in Italy (81 vs 80%), number of years lived in Italy (8.09 vs 8.31) and Socio-Demographic Index (5.15 vs 4.59%). Children’s Performance (in Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests are shown in Figure 1. The results overall showed significant differences between patients and controls in inhibition and planning (p=0.001 and 0.001 respectively) and in verbal tasks that involved Executive Functions more (i.e. phonological fluency) (p=0.004). The poorer verbal performance of children with SCD was not associated to visible lesions 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

Summary/Conclusions: Our results suggest that complement dysregulation is evident in asymptomatic SCD patients with increased HbS levels, an important tool in everyday clinical practice. APC activation during a painful crisis and the role of hydroxyurea need to be further investigated in larger series validating the role of different functional assays. Effective inhibition of complement activation in vitro is promising for future studies in selected patients.

PB2144

COMPLEMENT ACTIVATION IN PATIENTS WITH SICKLE CELL DISEASE IS ASSOCIATED WITH HIGHER HBS LEVELS

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1Hematology Department - BMT Unit, G. Papanciolo Hospital, 2Adults Thalassemia and Sickle Cell Disease Unit, 2nd Department of Internal Medicine, Aristotle University of Thessaloniki, Hippokration General Hospital of Thessaloniki, Thessaloniki, Greece

Background: Older studies have suggested activation of the alternative pathway of complement (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 activated serum. Normal human serum (NHS) was used as a negative control and lipopolysaccharides (LPS)-incubated normal serum as a positive control. All samples were tested in triplicates and twice. Eculizumab containing serum (ECU) was used in triplicate per patient within 60 minutes after the start used to test complement blockade by eculizumab in the modified Ham test.

Results: We studied 26 SCD patients (36±11 years of age, 12 male:14 female, 8 HbS/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 anti-thrombotic treatment. No differences on previous studies and other controls, percentage of non-viable cells higher than 20% was considered a positive modified Ham test, indicating increased APC activation in 4 SCD patients (Figure 1). Positive patients had significantly increased HbS levels (80.5±11 vs 61.8±16%, p=0.037), while 2/4 were homozygous (HbS/S) and 2/4 heterozygous (HbS/B-Thal). The majority (75%) were not on hydroxyurea. No significant difference was found regarding age, gender, platelets, white blood cells, Hb, HfB, 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. Effective inhibition of complement activation in vitro is promising for future studies in selected patients.
was a factor of the lower performance in this task. Figure 1. Children’s Performance (in Z scores) at Visuo-Spatial, Boston Naming, Phonological Fluency and Semantic Fluency Tests. P-values: Visuo-spatial intelligence: not significant (ns); Boston naming: ns; Phonol-Fluency: 0.004; Semantic fluency: ns.

at home to manage the onset of an acute crisis and the top 5 home strategies reported were: prescription pain medication (15%), sleep/rest (15%), apply heat using heating pad/blanket/bath/shower (13%), increase fluid intake (12%), and finally avoid stress (9%). Further it is clear, that people living with SCD are motivated to try a new therapy that could provide “significant relief” and “prevent symptoms from happening” due to their SCD.

Summary/Conclusions: The survey collected feedback about topics for which the patient is the best source of information. It is obvious that people with SCD are willing to self-medicate by subcutaneous injections and that there is a need for new tools and medications. With support from the answers from the survey, specific aspects will be considered while designing a first clinical study for subcutaneous sevuparin/DF02 administration to treat early symptoms of painful crisis in an at-home setting.

Table 1.  

| Number of respondents | Age       | Female | Male | SCD diagnosis | Ethnicity   |  |
|-----------------------|-----------|--------|------|---------------|-------------|  |
|                       | 70        | 47-48  | 67   | 80%           | African American/African descent | 97%  |
|                       |           |        |      | 30%           | Other       | 3%  |

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.modustopatientssurvey.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 to address these areas was conducted.

Summary/Conclusions: The survey collected feedback about topics for which the patient is the best source of information. It is obvious that people with SCD are willing to self-medicate by subcutaneous injections and that there is a need for new tools and medications. With support from the answers from the survey, specific aspects will be considered while designing a first clinical study for subcutaneous sevuparin/DF02 administration to treat early symptoms of painful crisis in an at-home setting.

PB2147  
LONG-TERM USE OF HYDROXYUREA IN CHILDREN AND ADOLESCENTS WITH SICKLE /BETA THALASSEMIA
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Background: Hydroxyurea (HU) has lately been used in the treatment of patients with severe sickle cell disease (SCD). Despite documented benefits on laboratory and clinical parameters in SCD patients, there are few reports about drug’s long-term safety and efficacy in pediatric patients with SCD – even more so in the rare patient subgroup of sickle/beta thalassemia.

Aims: A prospective, long term evaluation of HU efficacy and safety in children and adolescents with sickle/beta thalassemia (S/b thal).

Methods: Ten patients with S/b thal aged 3.5-18 years were followed for a 6 year period (Jan 2011- Dec 2016), HU was given at a daily dose that ranged from 10 to 20 mg/kg, with a mean of 14.1 mg/kg. Laboratory follow-up consisted of WBC, Hb, Ht, RBC, reticulocyte count and PLT count measured every 2 weeks until dose escalation to a stable dose, biochemistry assessed every 2 months and Hb F measured every 2-3 months. Patients were clinically evaluated prior to HU treatment and every 12 weeks during the study period. Evaluated data on clinical course included frequency of vaso-occlusive crises, hospitalizations and transfusions, as well as presence of severe clinical events. Hematologic toxicity of hydroxyurea was defined as a more than 20% decline from baseline in Hb, as an absolute neutrophil count of less than 1,000/μl and/or a PLT count of less than 80,000/μl. Moreover, presence of alopecia, rash, skin hyperpigmentation or headache was reported as drug-related toxicity.

Results: A significant reduction in vaso-occlusive crises as compared to prior to HU treatment was noted (median: 1 episode per year before HU, range: 0-2.5 vs median: 0.24 episodes per study year after HU, range: 0-1.33, p=0.011). A significant reduction in hospitalizations was also reported (median: 1 per year before HU, range: 0-3.2 vs median: 0.16 per study year after HU, 0-0.83, p=0.005). None of the patients presented with severe clinical events such as acute chest syndrome, avascular bone necrosis, stroke or splenic sequestration during the study period. With regards to hematological parameters, a significant increase in HbF (10.2±6.5% vs 16.6±7.1% p=0.02), MCV (66.1±3.9fl vs 79.3±8.4fl, p<0.001) and MCH (20.9±1.2pg vs 25.3±2.2pg, 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 short term 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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IN VITRO MODEL OF SICKLING REVERSAL USING ERYTHROCYTE SUSPENSIONS

Background: Erythrocyte sickling and adhesion are favoured by cellular dehydration, which increases the rate of hemoglobin polymerization and cell sickling. Potassium ion (K+) co-transport and calcium-activated potassium channel (Gar- dochans) mediate erythrocyte dehydration in sickle cell disease and β-thalassaemia. We investigated the in-vitro and in-vivo effects of various concentration of K+ ions in physiological solutions (PSS) as well as in cocos nucifera water (CNw) which is known for its natural high potassium content and isotonicity.

Aims: This study was aimed at ascertain the efficacy of high potassium isotonic solutions in rehydrating sickling cell and possibly reversing the sickling phenomenon in vivo and in vitro situations.

Methods: 1. Erythrocytes from twenty sickle cell anaemia (SCA) as well as healthy subjects were studied. One part was treated with sodium metabisulphite (Na2S2O5) solution to induce maximum sickling as controls while the other was subjected to different high concentrations of K+ in PSS as well as Cocos nucifera water (40mM, 80mM and CNW - 65mM/L) respectively. The procedure was repeated for the normal HB AA-subjects. Also, both groups of subjects were given 10ml/kg body weight of coconut water to drink as a single dose for the in-vivo experiment. Blood samples were collected longitudinally before and after the oral ingestion, at 1hr and at 24hrs for analysis of red cell indices as well as stained blood films used to ascertain the percentage sickled erythrocytes 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, respectively) to about 2% with Cocos nucifera and 10% with 80mM K+PSS. The count in 40mM K+PSS was not statistically significant. In both HB AA and SS subjects, MCHC was moderately stable when compared with the index solution sample (P>0.05, respectively) while MCHC increased significantly in both groups as early as 1hr and sustained till the 24th hour. MCHC was equally raised in the in-vitro samples (P=0.05, respectively). The morphology of red cells also indicated a lesser count of sickled red cells after the oral ingestion.

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 haemoglobin concentration (MCHC) and thereby restoring the normal red cell shape. We suggest a probable pharmacological value of the cocos nucifera water as well as other formulated high potassium but isotonic fluids in SCA management.
PB2151
DELAYED HAEMOLYTIC TRANSFUSION REACTIONS: A MASQUERADE OF SICKLE CELL COMPLICATIONS
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1Haematology, Croydon University Hospital, London, United Kingdom

Background: Patients with sickle cell disease (SCD) may require repeated red blood cells (RBCs) transfusion, putting them at risk from minor blood group alloimmunization and the development of delayed haemolytic transfusion reactions. A recent report documented a prevalence of DHTR syndrome in patients with SCD.

Aims: To report a prevalence of recognized DHTR syndrome in patients with SCD.

Methods: We reviewed the cases of DHTR in SCD patients in a 5-year period (2010-2016). A total of 10 patients had a clinical picture compatible with DHTR and underwent treatment with high dose steroids, intravenous immunoglobulins (IVIG) or erythropoietin. Any patient received Rituximab.

Results: The most common indications for transfusion were anemia due to vasoocclusive sickle cell crisis or preoperative anaemia optimization. The cohort received partial exchange transfusion and phenotypically matched RBCs. Before transfusion the median of Hb level was 69 g/L (baseline range 80g/L) and the nadir at haemolysis episode was 38 g/L. Ht was 21.9%, WBC was 17.3 × 10⁹ cells/L and mean LDH 1290 IU/L. The median time to develop DHTR was seven days after the transfusion and approximately 6 days after the surgical interventions (range: 4–12 days) and all cases presented with symptoms of anaemia, jaundice, tiredness and tachycardia. The median age was 29 years with female predominance (6:4). Blood cultures were negative in 80% of patients and only positive in 2 cases. 30% of patients tested positive for viral infection on PCR. Mortality rate in our series was low (zero). Pain episodes and other complications associated with DHTR was treated as required and four cases were successfully monitored in HDU. One patient required noninvasive ventilations and inotropic support. Two patients received RBC transfusion as part of protocol. Possibly as their presentation 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 hemolytic reaction. Patients in whom the diagnosis of DHTR is missed may receive repeat transfusions, which may contribute to the complications associated with SCD. The use of more extensive phenotypic matching of blood and minimizing RBC transfusion help to prevent DHTR. The present study emphasizes the importance of early recognition of symptoms and signs in correlation with a recent history of RBC transfusions, as DHTR can be a potentially life-threatening complication.

PB2152
HBS MONITORING ON TOSOH G8 IN VARIANT HBA1C MODE IN CASE OF URGENT RCE
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Background: Pre- and post-transfusion HbS levels are used to document the efficacy of red blood cell exchange (RCE) in patients with sickle cell disease (SCD). In case of urgent RCE a 24/7 STAT analysis, with the ability to identify and quantify hemoglobin (Hb) S, is warranted.

Aims: We evaluated the use of Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 (Tosoh Europe, Amsterdam, The Netherlands) for this purpose, using the variant HbA1c mode. Results were compared to our routine CZE Minicap Flex Piercing (Sebia, Lisses, France).

Methods: Within- and between-run imprecision were assessed using a sickle cell trait and a sickle cell anemia sample, aliquoted and stored at -80°C, twice using the variant HbA1c mode. Results were compared to our routine CZE Minicap Flex Piercing using 32 whole blood left-over HbS samples (HbS range: 9% - 93%). Data analysis was performed using Microsoft Excel Analyze-it version 4.6.5. Comparisons were made using the Student’s t-test. Differences were considered as statistically significant if the P-value was <0.05.

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 0.998 (>0.95) and a slope and intercept of 0.94 (95% CI: 0.92-0.96) and 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.
Summary/Conclusions: In SCD male and female seem to show a comparable risk in developing cardiac complication, although compared to females, males showed a significant lower risk of developing LV hypertrophy. There are no specific guidelines for SCD patients and, as a consequence, the cardiovascular follow-up is conformed to that one of thalassemia patients (complete cardiac evaluation performed annually for both genders). Our data not support a different follow up time based on the gender.

PB2154

SICKLE CELL PAIN IN CHILDREN: TARGETS FOR ADMINISTRATION OF ADEQUATE INITIAL ANALGESIA

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Background: Acute pain is a hallmark presentation in sickle cell disease (SCD) and frequently requires attendance to the emergency department (ED).

Aims: Here we report our findings following a complete retrospective audit cycle, documenting the timeliness of analgesia administration and post-treatment pain review as per National Institute of Clinical Excellence and College of Emergency Medicine guidelines, in children with SCD presenting to a single inner city London ED over a 14 month period.

Methods: In 2014, we evaluated 48 patient records of children presenting to the ED, with respect to mild, moderate and severe pain scores, time of analgesia administration and pain review. Completing the audit cycle, 97 records were re-audited in 2015. A total of 145 admission records were evaluated.

Results: In 2014 the ED met CEM criteria for the timeliness of analgesia administration in 100% of severe and 95% of the moderate pain category; however fell 33% short of NICE standards. Pain review was poorly performed, identifying an area for improvement. Proportions meeting the aforementioned criteria fell significantly in 2015, except review of moderate pain, which increased by 25%.

Summary/Conclusions: We conclude CEM guidelines promote timely administration of analgesia in patients with severe pain; however mild pain may be overlooked. NICE avoids this discrimination. Thus we recommend combining the mild and moderate pain categories to acknowledge the fluctuating nature of sickle pain and its tendency to rapidly escalate. In addition, we reiterate the need for regular pain reviews. This is important in ensuring analgesia is closely titrated to pain level.

PB2155

DIAGNOSTIC CHALLENGES IN A POPULATION WITH INCREASED IMMIGRATION: HEMOGLOBINOPATHIES IN THE NEW CENTURY

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Background: The diagnosis of hemoglobinopathies (Hppts) has changed in recent years due to immigration, with an increase in structural Hppts. In our region, Asturias, population census is 1,061,756 inhabitants; 48,097 out of them are immigrants.

Aims: Review the incidence of structural Hppts and thalassemias in our region in the last 10 years.

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 abnormal Hb patterns in the HbA1c test or suggestive results of thalassemia on hematology test. Studies were performed by high-performance liquid chromatography (HPLC) with the Variant Hemoglobin Testing machine of Bio Rad, and Hb electrophoresis with Paragon plates until May 2013. Since then, it has been performed by capillary electrophoresis (CE) with the MINICAP System of the laboratory.

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 390; 337 were β or δβ (86.4%); 54% came from Spain. The cases of β-thalassemia were 5 intermediate, 3 major, 1 β+-hemozymoyte 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 6.4-6.5), in 3 of intermediate (11.4 g / dL, range 6-10), and in 197 of minor (10.87 g / dL, range 6.9-9.1). - Structural Hppts 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=5). 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 (7.94g / 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 Hppts, 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 hematology results (Figure 1).
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=80) obese. All patients received single-unit UCBT after a myeloablative conditioning regimen. Diagnosis was acute lymphoid leukemia in 70% (n=363) and acute myeloid leukemia in 30% (n=154). Median age at UCBT was 7.4 years (range 2-19.6). Cytomegalovirus (CMV) serology was positive in 45% patients; 60% of patients were male. Most patients (92%) were in complete remission at UCBT. Median follow-up was 52 months (range 2-201). Total body irradiation (>6 Gy) was used in 58% of cases; antithymocyte globulin (ATG) in 68% of cases. Median infused total nucleated cell (TNC) dose was 4.2x10^7/Kg (0.3-17.8); 56% of patients received a graft with 0-1 HLA mismatch donor. Four-year overall survival (OS), leukemia-free survival (LFS) and disease-free survival (DFS), chronic GVHD, neutrophil engraftment, NRM and chronic GVHD between the 4 groups identified according to BMI was identified. Conversely, acute GVHD was 44.3% (33.3-58.8%) for underweight, 36% (31-41.8%) for normal, 26.2% (18.1-38.3%) for overweight and 23.3% (14.7-37.1%) for obese (p=0.03). Among patients underweight who experienced acute GVHD (n=27), 37.5% had grade III/IV acute GVHD with gut involvement. In multivariate analysis, infused TNC dose>4.2x10^7/Kg was associated with higher neutrophil engraftment (HR=1.46, CI 95% 1.19-2.78, p<0.001) 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 was associated with higher 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.
Methods:  for ASCT.

and pulmonary toxicity) after the two conditioning regimens (CFM-TBI
tions and non-neoplastic complications (grade 3-4 infections, cardiovascular
usual conditioning until year 2000.

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. How-
ever, 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: 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. Pretransplant conditioning regimen consisted of 30 mg/m² of Flu for 5 days (total 150 mg²/m²), 4 g/m² of AraC for 2-4 days (divided by 2 daily, total 8-16 g/m²) and 50mg/kg of Cy for a day. Four gray of TBI was used for all CB transplant recipients, whereas 2 gray of TBI was used in other stem cell sources. Using Flu/high-dose AraC/Cy +/- TBI was well tolerated with acceptable low toxicities and was sufficient to allow donor cell-engraftment post allo-SCT for elderly or frail patients with hematologic malignancies. Longer follow up and another prospective study enrolling more patients regarding non-neoplastic complications are required to evaluate the eventual survival benefit by reducing relapse.

Table 1. Patient’s characteristics.

<table>
<thead>
<tr>
<th>Age (years)</th>
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<tr>
<td>Complications</td>
<td>Infections</td>
<td>Other</td>
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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 (CYCLOPHOS-
PHAMIDE - 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 BEAM (a combination of carbustine (BCNU), etoposide, cytarabine and melphalan) although we also have a large experience with cyclophosphamide (CFM)-total body irradiation (TBI) since this was the usual conditioning until year 2000.

Aims: To analyze the cumulative incidence of secondary neoplastic complications (grade 3-4 infections, cardiovascular and pulmonary toxicity) after the two conditioning regimens (CFM-TBI vs BEAM) for ASCT. Methods: We performed a retrospective analysis of patients with NHL that 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 autografted patients were analyzed. Patient’s characteristics are in Table 1. The median follow up since ASCT was 73 months (0 – 274 months). Thirty-one percent (n=33) of patients were conditioned with CFM-TBI. The overall 5-years survival (OS) was 68.3% (58-77% - CI 95%) and the 5-year disease free survival (DFS) was 52% (42 61% - CI 95%). There were no differences regarding OS and DFS between the two conditioning regimens. The 5-years cumulative incidence (CI) of relapse was 0.48 (0.37-0.57. CI 95%). We detected 10 secondary neoplasm (myelodysplasia n=1, skin carcinoma n=2, lung carcinoma n=3, oropharageal carcinoma n=1, intestinal adenocar-
cinoma n=1, renal neoplasia n=1, bladder neoplasia n=1). The median time for the neoplastic event was 10.5 years (0-18.5 years). The CI of secondary neo-
plasias (2nd neoplasia) at 10 years was 10% (1-20%, CI 95%) and at last point of follow up (18.5 years) was 40% (13%>63%, CI 95%). There were no differences in the CI of 2nd neoplasias between BEAM and CFM-TBI. Non-neoplastic complications were present in 10% of patients (n=11). Three cases were infections grade 3-4 related to ASCT. Six cases had cardiac complications (5 acute coronary syndrome, 1 myocardiacopathy) and 2 had pulmonary toxicity. The CI of non-2nd neoplastic complications at 10 year was 10% (1-25%, CI 95%). No differences were detected between the two conditioning regimens regarding non-neoplastic complications. (see Figure 1).

PB2160

THE MANAGEMENT OF RELAPSED HODGKIN’S LYMPHOMA AFTER
HAPLOIDENTICAL STEM CELL TRANSPLANTATION: DONOR
LYMPHOCYTE INFUSION AND BRENTUXIMAB.
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Background: Hodgkin’s lymphoma, is an heterogeneous malignancy wich is possible to cure. For those patients who relapse, chemotherapy followed by an allogenic transplant (alloTPH) has been the standard of care. Allogeneic transplantation (alo-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 aloeSCT 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 hapl-
loid donor haematopoetic 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 lym
phoma. In our series, patients conditioned with BEAM or CFM-TBI had a com-
parable incidence of neoplastic and non-neoplastic events.
22nd Congress of the European Hematology Association

Methods: We studied 127 adult patients who underwent ASCT followed by LEED or MCEC as the conditioning regimen against chemo-sensitive ML at four institutions in Japan between 1997 and 2015. Any type of pathologic stage III, with adequate evolution after treatment.

Results: The median age was 32 years (21-60). 44% (8 patients) relapsed. The donor was a family member (REL) in 17 patients (54.8%), unrelated (MUD) in 14 patients (45.2%). The hematopoietic cell transplantation morbidity 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. 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 relapse, patients developed t-AML (n=19) (61.3%), t-PH+ ALL (n=1) (3.2%), or t-MDS (n=11) (35.5%). Molecular abnormalities were detected in 7 (46.7%) out of 15 evaluable patients: 16.7% was favourable risk (n=3), 27.8% was intermediate risk (n=5) and 55.5% was adverse risk (n=5). The disease status at transplant was as follows: complete remission (n=13) (42%), refractory disease (n=1) (3%), resistant disease (n=3) (10%). 6 patients received conventional chemotherapy in 14 cases (45.2%), azacytidine in 11 cases (35.5%), 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 morbidity 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 CBR/AML (1), ITD FLT3 (2), inv(16) (1), NPM1 (2), NPM1/ITD FLT3 (1). Karyotype aberrations were found in 18 (64.3%) out of 28 evaluable patients: 16.7% was favourable risk (n=3), 27.8% was intermediate risk (n=5) and 55.5% was adverse risk (n=5). The disease status at transplant was as follows: complete remission (n=13) (42%), refractory disease (n=1) (3%), resistant disease (n=3) (10%). 6 patients received conventional chemotherapy in 14 cases (45.2%), azacytidine in 11 cases (35.5%), 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 morbidity 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. 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PB2163

IMPROVEMENT IN BIVENTRICULAR CARDIAC MECHANICS NOTED IN PATIENTS UNDERGOING MYELOABLATIVE AUTOLLOGOUS-HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR AL AMYLOIDOSIS

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Background: Primary amyloidosis (AL) is characterized by extracellular deposition of insoluble protein fibrils often with multisystem organ involvement. The Mayo staging model for determining prognosis in patients with cardiac amyloidosis takes into account troponin, NT-proBNP, and serum free-light chain difference in order to stage patients prior to undergoing autologous hematopoietic stem cell transplant (Auto-HCT). Since amyloidosis often involves the kidneys, serum biomarkers that require renal clearance are less reliable in the setting of significant renal dysfunction. 2D-echo and strain imaging offer non-invasive modalities for identifying early cardiac changes independent of renal function. These changes may also precede symptom improvement as assessed by NYHA classification.

Aims: Our hypothesis is that strain imaging is a feasible biomarker for cardiac response after Auto-HCT in AL amyloidosis.

Methods: Seven patients with biopsy-proven AL amyloidosis who were treated with a Melphalan based myeloablative regimen and Auto-HCT were evaluated retrospectively. Each patient underwent 2D-echo up to 36-days prior to treatment followed by repeat 2D-echo within 14-months. Strain imaging was performed using Echoclinics®. Chart review was conducted to determine associated NYHA functional classification and Mayo staging. Statistical analysis was performed using SPSS.

Results: Of the 7 patients studied, 3 were Mayo stage I, 2 stage II, 1 stage III, and 1 stage IV. The median follow-up from transplant was 47.4 months. There was one death at 20.4 months. The mean NYHA classification at baseline was 2.3 (range 1-4) and at follow-up was 1.8 (range 1-2). There was no significant change in left ventricular ejection fraction (LVEF) (Figure 1).

Summary/Conclusions: We demonstrate that there is a clinically meaningful improvement in cardiac mechanics one year after Auto-HCT, despite no alteration in LVEF. This metric may prove useful in assessing organ response, especially when serum biomarkers are less reliable. Changes in left ventricular GLS occur independent of pre-transplant Mayo stage, although prospective studies are needed for confirmation. We further believe that improvements in RVFWS may predict clinical improvement.
expensive instrumentation, high reagent costs, and poor reproducibility between technicians and laboratories.

Aims: We developed and assessed an instrument performance of a newly-developed image-based microscopic cell counter (ADAM II®) for enumeration of CD34+ cell and its viability.

Methods: We 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 of ADAM II® with expected 14.77-172.06 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%. The linearity of CD34+ count was confirmed over a range of dilutions (0.58-280 cells) of sample. Linearity was 1.004. 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 between the two flow cytometers.

Summary/Conclusions: The newly developed image-based microscopic cell counter (ADAM II®) appears to be suitable for quantification of CD34+ cell and its viability of fresh or cryopreserved PBSCs or CBs.

PB2166

EXTRACORPOREAL PHOTOPHERESIS IN STEROID-DEPENDENT OR REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE

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Background: Extracorporeal photopheresis (ECP) has been incorporated in the management of graft-versus-host disease (GVHD) post allogeneic hematopoietic cell transplantation (alloHCT) in many centers. The introduction of ECP as an early second-line treatment in steroid-dependent or refractory patients with acute GVHD (aGVHD) remains under study. The rationale of its early use is based on the low incidence of complete responses to corticosteroids and the profound immunosuppression caused by traditional secondary treatments.

Aims: Based on our long-lasting experience in chronic GVHD, we aimed to prospectively assess the role of ECP in this high-risk population.

Methods: We enrolled consecutive patients with steroid-dependent or refractory grade (gr) II-IV aGVHD post alloHCT from January 2013 to August 2016. All patients with unrelated or haploidentical donors received thymoglobulin (ATG) 5mg/kg as prophylaxis. Post-transplant GVHD prophylaxis included cyclosporine – mexitretaxate in myeloablative and cyclosporine – mycophenolate mofetil in reduced intensity or intensity regimens. ECP was commenced after assessment of response to 5 days of steroid treatment according to our protocol: 2-3 sessions/week for 1 month, 1 session/week for 3 months, evaluation of response and 1 session/month for 6 months.

Results: We studied 20 patients, aged 35 (18-65), post alloHCT with myeloablative (14), reduced toxicity (4) and intensity (2) conditioning, from sibling (10), unrelated (8) and haploidentical (2) donors. Disease risk index was high (10), intermediate (9) and low (1). Acute GVHD was observed at day +17 (8-50) in 15 patients, late-onset at +130 (110-160) in 4 patients and induced at +38 post donor lymphocyte infusion in a case of aGVHD post alloHCT. The majority of patients (16/20) presented partial (6), very good (9) or complete (1) response to ECP. With 9.3 (1.8-54.7) months of follow-up, immunosuppressive treatments were reduced in 10/20 and ceased in 1 patient. Clinically significant bacterial infections were found in 17 patients, fungal in 2, CMV and EBV reactivation in 14 and 13 respectively and other viral in 5 patients. Cumulative incidence (CI) of chronic GVHD was 77.4 at 1-year, 1-year CI of aGVHD-related mortality was 58%, 1-year overall survival (OS) was 53% and significantly increased to 90% in steroid-dependent versus refractory patients (76% vs 36%, P=0.014). Reduction of immunosuppression (p=0.026) and steroid dependence (p=0.023) were associated with improved OS, irrespectively of other factors.

Summary/Conclusions: Our study supports that ECP should be considered early in the course of steroid-dependent or refractory aGVHD, before significant irreversible end organ damage has been established. Optimal timing of intervention, frequency, duration and tapering schedule of ECP need to be investigated in future studies.

PB2167

RAPID RECONSTITUTION OF NK1 CELLS IS ASSOCIATED WITH THE LOWER INCIDENCE OF GRAFT-VERSUS-HOST DISEASE AFTER ALLOGENIC TRANSPLANTATION

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Background: The balance between immunostimulation and immunoregulation in T cell immunity is achieved by a Th1/Th2/Th3/Tr1 and CD4+CD25+ regulatory T (T reg) cell paradigm.

Aims: We investigated the production of type1 (IFN-gamma, NK1), type2 (IL-13, NK2), type3 (TGF-beta, NK3) and regulatory cytokines (IL10, NKr) from human peripheral blood to discuss the cytokine paradigm of NK cells in human allogeneic hematopoietic stem cells transplantation (allo-HSCT).

Methods: Forty patients undergoing haploidentical (n=27) and HLA-identical sibling (n=13) allo-HSCT between August 2009 and December 2009 were enrolled in this analysis after being originally selected using a protocol exploring the association of reconstituted donor derived NK1/NK2/NK3/NKr cells to GVHD and CMV reactivation.

Results: Expansion of NK2 and NK3 were found post allo-HSCT compared to healthy donor. The levels of NKr reconstituted to donor’s level since day 15 post allo-HSCT, and the levels of NK1 in recipients post transplantation were consistently lower compared to donors’ levels until day 60 post allo-HSCT. Multivariate analysis showed that the higher levels of NK1 by day 15 was associated with lower overall acute GVHD (HR 0.157, 0.039-0.642, P=0.019) as well as II-IV acute GVHD (HR 0.260, 95%CI, 0.064-1.053, P=0.059). Meanwhile, the higher levels of NK1 by day 15 correlated with lower CMV reactivation (HR 0.011, 0.005-0.348, P<0.003).

Summary/Conclusions: These results indicate that rapid reconstitution of NK cells; especially NK1 cells would be helpful to prevent the development of graft-versus-host disease as well as CMV reactivation after allogeneic transplantation.

PB2168

BORTEZOZIM 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 immune suppression 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 included 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>
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<th>Age at allo-HSCT (y)</th>
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Table 1

Summary/Conclusions: Our study shows that Bortezomib is a promising therapeutic option for refractory post-transplant autoimmunity with high tolerance and no related toxicities.
Background: The infused cell count in cord blood transplantation (CBT) is an important element for engraftment; however, the number in the prior reports has been based on the pre-thaw cell count. Therefore, the association between post-thaw cell count and engraftment rate, especially in pediatric patients, is unclear.

Aims: The Aim of this study is to reveal the association between post-thaw cell count and engraftment rate in pediatric patients in the setting of CBT at our institution.

Methods: We retrospectively reviewed the medical records of 78 patients who underwent CBT between June 1998 and April 2016. We excluded the cases of CBT that required rescuing after engraftment failure.

Results: Underlying disease was acute leukemia (AL) in 63 (ALL: 38; AML: 25) patients, chronic myeloid leukemia in one, malignant lymphoma (ML) in two, and myelodysplastic syndrome (MDS) in three, aplastic anemia in one, and others (such as primary immunodeficiency) in eight. In terms of conditioning regimens, myeloablative conditioning was administered to 62 patients and reduced intensity conditioning was administered to 16 patients. The median age at CBT was 3 (range: 0–19) years, and the median follow-up period was 896 (range: 47–6236) days. The engraftment rate was 84.6%, primary engraftment failure was observed in 11 patients (AL seven, ML one, MDS one, neutroblatoma, one, and others, one) and secondary graft failure was observed in one patient (severe congenital neutropenia). The engraftment rate was 55.1%, and 32 patients had died (cause of death: progressive disease in 19 patients). We analyzed the data on 34 patients of whom both of pre- and post-thaw CD34+ cell counts in the cord blood samples were available. The median post-thaw CD34+ cell count was 1.60 × 10^9/kg in the patients who achieved engraftment and 1.01 × 10^9/kg in the patients who did not achieve engraftment. No statistically significant difference was observed between these two groups (p=0.30). When we defined the cut-off value of the pre-thaw CD34+ cell count as 1.2 × 10^9/kg in the patients who were infused with CD34+ cells more than the cut-off value, the sensitivity and specificity of graft failure was 79.3% and 60%, respectively. When we defined the cut-off value of the post-thaw CD34+ cell count as 0.7 × 10^9/kg in the patients who were infused with CD34+ cells more than the cut-off value, the sensitivity and specificity of graft failure was 96.6% and 40%, respectively.

Summary/Conclusions: We concluded that the risk of graft failure is more precisely predicted by the post-thaw than pre-thaw CD34+ cell count and that if the median post-thaw CD34+ cell count is more than 0.7 × 10^9/kg, the risk of graft failure is very low.
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 analyses, 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-engraftment (3/31, 9.7% vs 31.5%, p=0.26),leukaemia-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 significantly worse than those of AL, and MDS is strongly associated with high NRM even with RIST. This indicates that we should pay more attention to NRM in MDS.

### PB2173

FACTORS PREDICTING GRAFT-VERSUS-HOST DISEASE-FREE, RELAPSE-FREE SURVIVAL AND OUTCOMES AFTER REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION FOR ACUTE LEUKEMIA OR MYELODYSPLASTIC SYNDROMES

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**Background:** Reduced intensity allogeneic stem cell transplantation (RIST) is now commonly applied for elderly patients with acute leukemia (AL) or myelodysplastic syndromes (MDS). However, the factors affecting graft-versus-host disease-free, relapse-free survival (GRFS) and overall survival (OS) remain obscure.

**Aims:** To identify such factors and to clarify the clinical significance of RIST with various graft sources, we retrospectively analyzed patients with AL or MDS who received RIST in our hospital.

**Methods:** The study included patients with acute myeloid leukemia (n=73), acute lymphoid leukemia (n=31) or MDS (n=25), who received fludarabine (Flu)/melphalan (Mel)-based RIST between 2004 and 2015 as the first treatment for AL or MDS: 60% (vs 38%, p=0.005) vs 62%, (p=0.012), disease (AL vs MDS: 60% vs 28%, p<0.001) and rDRI (L/I vs H/V: 65% vs 38%, p=0.003). On multivariate analysis, BM/PB (HR 2.0, 95% CI 1.0-4.0, p=0.039), MDS (HR 2.6, 95% CI 1.5-4.6, p=0.001) and H/V (HR 2.1, 95% CI 1.2-3.5, p=0.006) were associated with a worse GRFS. The 5-year OS, cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) were 55%, 36% and 18%, respectively. On univariate analysis, significant prognostic factors were hematopoietic stem cell transplantation-specific comorbidity index (HCT-CI) (score 0 vs >=1: 78% vs 48%, p=0.007), disease (AL vs MDS: 59% vs 40%, p=0.004) and rDRI (L/I vs H/V: 64% vs 43%, p=0.003) for the 5-year OS, donors (related vs unrelated): 53% vs 27%, p=0.005) and rDRI (L/I vs H/V: 27% vs 48%, p=0.005) for CIR, and age (<60 vs >=60: 10% vs 28%, p=0.021), donors (related vs unrelated): 8% vs 23%, p=0.034) and disease (AL vs MDS: 13% vs 36%, p=0.003) for NRM. On multivariate analysis, HCT-CI (HR 1.9, 95% CI 1.1-3.5, p=0.02) was associated with worse OS, so were H/V rDRI (HR 2.5, 95%CI 1.4-4.7, p=0.003) and MDS (HR 3.7, 95%CI 1.6-8.8, p=0.002) for CIR and NRM, respectively.

Summary/Conclusions: 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 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.

**Background:** Hemorrhagic cystitis (HC) is a serious complication occurring after allogeneic hematopoietic stem cell transplantation (HSCT) more frequent on haploidentical (haplo) HSCT, with an incidence of 10% to 70% (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 dose on 32 pts and 8 hours on all pts. We used SPSS V.23 to determine the cumulative incidence (CI) of HC.

**Results:** We performed 20 haplo-HSCT, of which 10 were males (1 was transplanted 3 times) and 8 were women. The mean age was 40 (range 16-64). The pts presented the following characteristics: 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 1, 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 OS and disease-free survival 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.
PB2175
OUTCOME OF CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS UNDERGOING NON-MYELOABLATIVE ALLOGENIC STEM CELL TRANSPLANTATION AFTER TREATMENT WITH THE BRUTON TYROSINE KINASE INHIBITOR IBRUTINIB
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Background: Although the Bruton tyrosine kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of CLL patients (pts), allogeneic hematopoietic stem cell transplantation (HCT) remains the only curative option for the underlying disease. Data on pre-transplant treatment of CLL with ibrutinib are very limited.

Aims: Here we present our experience of HCT in pts previously treated with ibrutinib.

Methods: 11 CLL pts (median age at HCT 57 years [n], range 52-66) treated between 2014 and 2016 in our unit with non-myoeloblastic (nma) HCT after ibrutinib were included. Ibrutinib treatment lasted median 4.03 months (range 1-28). Conditioning regimen was Fludarabin 30 mg/m2 on day -4 to -2 followed by 2 Gy total body irradiation. Disease status at HCT was Binet B (n=3) or Binet C (n=8). Two pts had Richter’s transformation (RT) diagnosed before nma-HCT. Ten pts were in partial remission (PR) at nma-HCT (PR1 n=4; PR2 n=3; PR3 n=2, PR4 n=1) while one was in first relapse. Donors were human leukocyte antigen (HLA) matched related (n=3, MRD) or HLA-matched unrelated (n=8, MUD). Pts received median 3 lines of therapy (range 1-6) including ibrutinib before transplantation. Classical cytogenetic analysis and fluorescence in situ hybridization (FISH) was carried out for every pt. Five pts had a deletion (del)(17p13) and one a del(11q22).

Results: The average overall survival (OS) for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 456 days (range 36-812) for those without del(17p13), p=0.98. OS was not significantly influenced by the stem cell source (MUD vs MRD, p=0.63) or remission status PR1 vs >PR1 (353 vs 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn’t reach significance (p=0.39). EFS was median 125 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p=0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our group (30% at 4y). The acute GVHD Grade 3-4 was present in 3 pts (27.2%).

Summary/Conclusions: The outcome in this small group is comparable to studies of CLL-pts undergoing nma-HCT without receiving ibrutinib upfront. Previous data from our unit (Hebenstreit et al., Leuk Lymphoma 2014) showed OS 51% and NRM 30% at 4y. Ibrutinib appears to be a feasible option in a transplantation setting, although further testing with larger numbers of patients and a longer follow up is required.

PB2176
SHORT-TERM CHIMERISM IN T-HELPER CELL SUBSETS AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION AND A longer follow up is required.
F. Ramdohr1,*, G.-N. Franke1, M. Jentzsch1, W. Pönisch1, C. Michael1, S. Heyn1, S.-Y. Wang1, G. Behre1, S. Leiblein1, S. Schwind1, D. Niederwieser1, V. Vucinic1, F. Ramdohr1,*, G.-N. Franke1, M. Jentzsch1, W. Pönisch1, C. Michael1, S. Heyn1, S.-Y. Wang1, G. Behre1, S. Leiblein1, S. Schwind1, D. Niederwieser1, V. Vucinic1
1Dept. Hematology & Clinical Oncology, University of Leipzig, Leipzig, Germany

Background: Although the Bruton tyrosine kinase (BTK) inhibitor ibrutinib significantly improves the prognosis of CLL patients (pts), allogeneic hematopoietic stem cell transplantation (HCT) remains the only curative option for the underlying disease. Data on pre-transplant treatment of CLL with ibrutinib are very limited.

Aims: Here we present our experience of HCT in pts previously treated with ibrutinib.

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Results: The average overall survival (OS) for all pts was 471 days (range 36-812) (Figure 1). The average OS of patients with del(17p13) was 379 days (range 66-628) compared to 456 days (range 36-812) for those without del(17p13), p=0.98. OS was not significantly influenced by the stem cell source (MUD vs MRD, p=0.63) or remission status PR1 vs >PR1 (353 vs 472 days, p=0.79). Non-matched CMV-Status (negative recipient and positive donor or positive recipient and negative donor) had an OS comparable to that of matched CMV-Status (p=0.73). Pts above the median age had a lower OS although this didn’t reach significance (p=0.39). EFS was median 125 days (range 26-628). Pts with or without a TP53 alteration had a similar EFS (p=0.91). Pts undergoing MRD-HCT had better EFS than those undergoing MUD transplantation (p=0.055). CMV-Status or age>median had no prognostic influence on the EFS (p=0.83 and p=0.39 respectively). Non-relapse mortality (NRM) was 32% at 10 months (Figure 1), which was consistent with a previous publication from our group (30% at 4y). The acute GVHD Grade 3-4 was present in 3 pts (27.2%).

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PB2177
ADIPOSE TISSUE CHANGES IN LYMPHOMA PATIENTS IN THE PERI TRANSPLANTATION PHASE
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Background: Abdominal Visceral Adipose Tissues (VAT) have been shown to have inflammatory activity and have been used to predict cancer outcomes. We hypothesized that the changes in VAT and subcutaneous tissues among stem cell lymphoma recipients in the peri-transplantation phase.

Methods: Institutional Review Board approved this retrospective study for adult patients (age>16 years) having B and T lymphoma who underwent Stem Cell Transplantation (SCT). Each patient was imaged by PET/CT scan pre-SCT and in the first 3 months post transplantation. A cross sectional image was analyzed at the level of the L3 to calculate VAT, SAT and Waist Circumference (WC). Data was analyzed by gender since body composition parameters differed significantly between the two categories in the literature.

Results: The study sample consisted of 91 patients [mean age: 37.7±13.5 years, n=52 (57%) males, n=39 (43%) females], 81 (87%) autologous SCT, 12 (13%) allogeneic SCT, median overall survival in months: 12 in males and 19 in females]. Death was observed in 6 (11.5%) males and 1(2.4%) female. Patient characteristics were similar across gender categories except for weights (kg) and Body Mass Index (kg/m2): 68.1 and 25.0 in males and 25.0 and 20.0 in males and females respectively. Changes from pre-SCT to 3 months post SCT revealed that VAT and SAT decreased with mean differences of 33±56 cm2 (p<0.01) and 7.0±36 cm2 (p=0.017) in males and 16±44 cm2 (p=0.001) and 4±14 cm2 (p=0.056) in females, respectively. Waist circumference decreased significantly with mean

Figure 1. 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. TAT, 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 underwent the second transplant - five patients - because of AML relapse, 2 - because of sepsis, 1 - graft failure. Out of re-transplanted 7 patients - 3 patients are alive - 2 patients with graft failure and one with post-transplant AML relapse in 2nd CR. Out of 93 procedures of unrelated donor transplantation there were 16 cases of death - assumed as non relapse mortality NRM (17%). There were 9 early deaths (before day +100) - 6 cases in patients with relapsed/refractory acute leukaemia without remission after conventional chemotherapy. These patients were subjected to sequential conditioning with cyclophosphamide. Active disease and highly active conventional chemotherapy. These patients were subjected to sequential conditioning with cyclophosphamide. Active disease and highly active conventional chemotherapy. These patients were subjected to sequential conditioning with cyclophosphamide.

Figure 1. Adipose Tissue Changes in the peri-transplantation phase presented by gender. TAT: Total Adipose Tissues; VAT: Visceral Adipose Tissues; WC: Waist Circumference

Summary/Conclusions: Based on these results we create working hypothesis that HLA class II haplotype may predispose to severe post-transplant infectious or/and non-infectious complications and affect the risk of NRM. Because small number of analysed patients and documented high frequency of these haplotypes in population, further analysis is required.

PB2179
HAPLOIDENTICAL STEM CELL TRANSPLANTATION WITH HIGH DOSE CYCLOPHOSPHAMIDE POST-TRANSPLANT IN HIGH RISK HEMATOLOGIC MALIGNANCIES: RISK FACTOR AND OUTCOME ANALYSES IN OUR CENTER
J. Viedma1,*, M. Saez-Perdomo1, M. Perera1, A. Suarez1, J. Lopez1, C. Medina1, L. Guerra1, M. T. Gómez-Casares1, H. Luzzardo1, T. Molero1, S. Jimenez1
1Hematology, Hospital Universitario de Gran Canaria Dr. Negrín, Las Palmas de Gran Canaria, Spain

Background: Allogenic hematopoietic stem cell transplantation (allo-HSCT) is an effective therapy for a variety of hematological malignancies. However, a lack of HLA-identical sibling donors or unrelated donors has restricted the application of allo-HSCT in hematological malignancies. Haploidentical HSCT (Haplo-HSCT) offers the benefits of rapid and nearly universal donor availability and, in the past decade, has been accepted worldwide as an alternative treatment for patients with hematological malignancies who do not have an HLA-identical sibling donor or who require urgent transplantation. Aims: The purpose of this study was to investigate the incidence, causes and factors influencing overall and transplant-related mortality after Haplo-HSCT. Methods: We analyzed all consecutive patients receiving Haplo-HSCT from family donors at our hospital from 2013 to 2016. The conditioning regimen used for the transplant was the Hopkins haplo protocol with high dose Cy (50 mg/kg on days 3 and 4) posttransplantation. We classified the patients before the Haplo-HSCT according to disease risk index (DRI), ECOG, Sorror score and EBMT risk score to evaluate the correlation between the physical state of the patients before the transplant and the survival (overall mortality (OM) and transplant-related mortality (TRM)). We used SPSS V.23 to calculate the cumulative Mortality incidence by the KM test and the Cox proportional hazards model.

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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 underwent the second transplant - five patients - because of AML relapse, 2 - because of sepsis, 1 - graft failure. Out of re-transplanted 7 patients - 3 patients are alive - 2 patients with graft failure and one with post-transplant AML relapse in 2nd CR. Out of 93 procedures of unrelated donor transplantation there were 16 cases of death - assumed as non relapse mortality NRM (17%). There were 9 early deaths (before day +100) - 6 cases in patients with relapsed/refractory acute leukaemia without remission after conventional chemotherapy. These patients were subjected to sequential conditioning with cyclophosphamide. Active disease and highly active conventional chemotherapy. These patients were subjected to sequential conditioning with cyclophosphamide. Active disease and highly active conventional chemotherapy. These patients were subjected to sequential conditioning with cyclophosphamide.

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Summary/Conclusions: Based on these results we create working hypothesis that HLA class II haplotype may predispose to severe post-transplant infectious or/and non-infectious complications and affect the risk of NRM. Because small number of analysed patients and documented high frequency of these haplotypes in population, further analysis is required.
ence between OM from the different states of ECOG (p=0.356) and DRI (p=0.07), however we found a statistically significant difference for ECOG (p=0.028) (Figure 1b) and Sorror (p=0.016). On a pairwise analysis of the OM we found no statistically significance for ECOG, and found a statistically significant difference between the patients with low-high DRI (p=0.01), intermediate-high DRI (p=0.001), ECOG 0-1 (p=0.046) and Sorror 0-5 (p=0.003). The multivariate analysis showed that ECOG 2 vs 0 (p=0.013, HR=4.6 95%), Sorror 2-3 vs 0-1 (p=0.041, HR=19.55) and Sorror 4-5 vs 0-1 (p=0.005, HR=282.48) were significantly related with a higher incidence of OM. Five patients died of infection (41.67%), 3 of disease progression (25%), 1 of relapse (8.33%) and 3 of other causes (25%). Six patients died of TRM (50%). The CI of TRM was 10.5% at 1 m and 31.6% at 3 m, 6 m, 1, 2 and 3 years (Figure 1c). When we analyzed the TRM depending on the different time status scores we only found a statistically significant difference between TRM incidence from the different states of ECOG (p=0.038) (Figure 1d) and no statistically significant difference for ECOG (p=0.386), DRI (p=0.372) and Sorror (p=0.372). The multivariate analysis also found statistically significant differences between ECOG 1-2 (p=0.018) and EBMT 1-5 (p=0.046), for Sorror we found a marginal statistical signification between 0-1 (p=0.052), 0-2 (p=0.052) and 0-5 (p=0.052), for DRI we found no statistically significant difference. On the multivariate analysis we found no statistically significant correlation between TRM and the physical status scores.

Summary/Conclusions: Despite the fact that Sorror, ECOG and DRI scores are widely evaluated to establish the risk of patients undergoing HSCT, in our experience ECOG remains a useful score for assessing the risk of TRM on patients receiving Haplo-HSCT. We think further studies with a larger sample would be necessary to confirm our results.

PB2180

A SIMPLIFIED METHOD OF CRYOPRESERVATION OF PERIPHERAL BLOOD STEM CELLS WITH OVER 10% GRANULOCYTE CONCENTRATION FOR LESS THAN 36 MONTHS

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Background: The long-term stability of cryopreserved peripheral blood stem cells (PBSCs) is an important concern for patients experiencing disease relapse. However, the quality of long-term cryopreserved PBSCs stored at -80°C by using simplified method has not been elucidated in detail. Cryopreserved PBSCs undergo cell damage and decrease in viability, and those containing granulocytes might influence cell loss. Aims: The aim of this study was to evaluate the effect of cryopreservation for less than 36 months and the number of granulocytes in the cryopreserved PBSC products on CD34+ cells.

Methods: We examined the effects of cryopreservation on the viability of CD34+ cells that were stored for less than six months and those stored for 7–24 months, and 25-36 months, and the change of CD34+ cell viability with higher granulocyte content. We also evaluated the correlations between the number of granulocytes in the cryopreserved PBSC products and the time to engraftment of lymphocytes or platelet. Informed consent was obtained prior to the procedure from all the patients following institutional guidelines.

Results: A total of 65 PBSC samples were collected. We compared three groups based on the cryopreservation period: (1) less than 6 months, (2) 7–24 months, and (3) 25-36 months. The median (range) viability of CD34+ cells after thawing was 81.8% (59.2–94.4), 80.5% (56.6–92.8), and 76.1% (54.5–89.6) in the three groups, respectively. No significant difference in the viability of the cells in either frozen period was observed (p=0.14, respectively). We compared the effect of granulocyte concentration (over 10% concentration against less than 10% concentration) on CD34+ cells viability. The median (range) viability of CD34+ cells containing >10% granulocytes was 76.6% (54.5–93.0%), and that for cells containing <10% granulocyte was 82.1% (59.1–94.4%), respectively. There was significant difference in the viability of CD34+ cells between the two groups (p=0.02, respectively). Second, we analyzed 81 autologous PBSC transplants after stored at -80°C by using simplified method. We analyzed the two groups based on the granulocyte concentration (over 10% concentration against <10% concentration). No significant difference in the days to leukocyte >1.0x10⁹/L and to platelet >20x10⁹/L in either granulocyte concentration was observed. However, the median (range) time to platelet >50x10⁹/L, containing >10% granulocytes was 27.2(12-87), and that for cells containing <10% granulocyte was 20.3(10-51), respectively. There was no significant difference in the day to platelet >50x10⁹/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 be safely stored for at least 36 months by using 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 platelet >50x10⁹/L between the two groups based on the granulocyte concentration (>10% concentration against <10% concentration) was observed. Thus, a lesser granulocyte content could give a more reliable graft with better quality. Further research is necessary to observe the effect of long-term cryopreservation period and granulocyte content on the viability of stored CD34+ cells.
PB2182

SUCCESSFUL AUTOLOGOUS STEM CELL TRANSPLANTATION AFTER VELCADE-BASED REFRACTORY MULTIPLE MYELOMA PATIENTS

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Background: The optimal induction treatment for Newly Diagnosed Multiple Myeloma Patients needs combinations with 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 proven efficacy in NDMM younger patients that have got at least a partial response (PR) after the induction therapy. There are few data about toxicity and response of ASCT in primary refractory patient that can obtain a response with LenDex rescue treatment.

Aims: Analysis of tolerance, response and overall survival of ASCT-candidates that are primary refractory to Bor-Based induction treatment.

Methods: Retrospective analysis of our database. From 2010 to Nov-2016, 53 ASCT-Candidates (for 1st or 2nd ASCT procedures) were included. Median Age for diagnosis was 62 (46-71). Median Age for ASCT procedure was 63 (46-72). 12 of these 53 patients (22.6%) were considered primary refractory and 41 candidates to 2nd Bor-Based induction treatment. 6 (13%) 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 (3). Median number of cycles administered: 6 (3-12). Of the other 9, 6 of them were considered candidate to intensificate treatment with high doses chemotheraphy 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-rescue ASCT-patients. Median age at ASCT: 62 (49-70). All of them received Melphalan 200 mg/m2 as conditioning treatment.

Results: Morbidity or mortality (M&M) (0%) of ASCT procedure in refractory patients is similar to non-refractory patients. After a median follow up of 46 months from diagnosis for all ASCT-candidates group, the refractory patients get an overall survival of 46.2 months (3-72 m). Any of them have relapsed yet. 2 of them are in biological relapse without need of treatment.

Summary/Conclusions: Patients refractoriness to induction may receive ASCT after a rescue treatment LenDex based, as is effective in this group conversion. 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 this 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 myeloablative with a reduced-toxicity conditioning (RT) regimen to decrease relapse rate after allogeneic stem cell transplant (allo-SCT) without increasing non-relapse mortality (NRM), has not been well established.

Aims: In this retrospective study at the American University of Beirut medical center (AUBMC) we aimed to evaluate the outcomes of patients who underwent allo-SCT with thiopeta, busulfan and fludarabine (TBF) as RT.

Methods: We included twenty fourconsecutivepatients with hematological malignancies who received TBF as conditioning forallo-SCTfrom January to December 2016. All patients and transplant characteristics are listed in Table 1. All patients received the myeloablative conditioning regimen consisting of thiopeta(5mg/kg/day) infused on day -7 and -6, fludarabine(30mg/m2/day) infused on day -5 to day -2, and busulfan(130mg/m2/day) infused on day -5 to day -3. All patients received 2.5mg/kg/day intravenous rabit antihuman-antibody mAb (ATG) on days -2 and -1. GVHD prophylaxis for patients transplanted from haploidentical donors consisted of post-transplant cyclophosphamide 50mg/kg/day on day +3 and +5, cyclosporine started at 3 mg/kg/day on day +6 and readjusted according to level, and mycophenolate mofetil 500mgx4/day started on day+6 to +28. Patients transplanted from matched related donor, received cyclosporine as of day +1.

Results: Twenty three patients(96%) engrafted, with 14 days (range, 10-18) as median time for neutrophil and platelet engraftment respectively. One patient who underwent haploidentical donor transplant with persistent disease for AML (karyotype 45, XY,-7) failed to engraft and died due to disease progression on day+22. After a median follow up of 10 months (range, 1-22) post-allo-SCT, the cumulative incidence of Gradel1-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 two (13%) died due to disease progression and two were successfully salvaged and are in complete remission (CR) with full donor chimerism (FDC) at last follow up. Two patients developed JC virus progressive multifocul leukoencephalopathy, one of them 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 TBF conditioning regimen appears to be safe,allows high rate of engraftment and low NRM rate among high-risk patients and can lead to a long-term disease control.

PB2184

COMPLETE REMISSION STATUS BEFORE AUTOLOGOUS STEM CELL TRANSPLANTATION AS PROGNOSTIC FACTOR IN PATIENTS WITH NON-HODGKIN LYMPHOMA

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Background: High dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) is commonly used for treatment of relapsed or refractory non-Hodgkin’s lymphoma (NHL), as well as for first-remission consolidation in patients with mantle cell lymphoma. Disease status before ASCT is variable and is unclear whether complete response before ASCT or after ASCT correlates with better survival.

Aims: To evaluate the prognostic effect of disease status before ASCT - complete remission (CR) vs partial remission (PR) - in a cohort of patients with NHL.

Methods: Retrospective analysis of patients with NHL treated with HDC and ASCT between 2007 and 2016 in a single institution. All patients received peripheral blood support after conditioning with BEAM regimen (carmustine 300mg/m2, etoposide 800mg/m2, Ara-c 1600mg/m2 and melphalan 140mg/m2). Response was assessed according to The Lugano Classification. The Kaplan-Meier method was used to estimate progression free survival (PFS) and overall survival (OS) and comparison between risk groups was performed by using the log-rank test. Univariate analysis was performed and significant predictors at the level of 0.05 were used to adjust a multivariate Cox regression model.

Results: We included 83 NHL patients, mainly males (72.3%) with a median age at diagnosis of 51 years (18-65). The most prevalent histological subtypes were diffuse large B cell lymphoma (53.0%), mantle cell lymphoma (36.1%) and follicular lymphoma (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/HADPICE (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 (86.6%) achieved a CR, 4 (5.1%) a PR and 5 (6.3%) failed to respond. Patients in CR before ASCT presented significantly longer PFS compared with those in PR (107.9 vs 44.0 months, p<0.01). Besides that, patients that obtained CR after ASCT also had longer OS and PFS compared with those in PR (107.9 vs 8.0 and 107.9 vs 7.3 months, p<0.001). However, these patients had significantly lower PFS compared to patients that continued in CR after ASCT (45.3 vs 107.9 months, p=0.041). Univariate analysis indicated that remission status prior to PRCT (CR vs PR) is a significant predictor of PFS after ASCT (HR 0.39; 95% CI 0.19-0.82, p=0.013). Multivariate Cox regression model showed that this factor retains prognostic value after adjustment for age, histological subtype, Ann Arbor stage and number of previous lines of treatment.

Summary/Conclusions: Our results highlight the relevance of the obtained CR after ASCT in the OS. Furthermore, we conclude that patients with NHL who are in CR before ASCT have a better PFS than those in PR before ASCT. Additionally, continued CR after ASCT may also be an important prognostic factor. Our results suggest that the use of more effective induction regimens in order to improve initial response may be advantageous in terms of clinical benefits post-ASCT.

**PB2185**

**AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MANTLE CELL LYMPHOMA: SINGLE CENTER EXPERIENCE**

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**Background:** Mantle cell lymphoma accounts for relatively small proportion (3%-10%) of non-Hodgkin lymphoma. High-dose chemotherapy (HDT) and autologous-stem cell transplantation (ASCT) has played a critical role in the treatment of mantle cell lymphoma. Regardless of that, mantle cell lymphoma remains largely a relapsing/remitting disease.

**Aims:** Our aim is to present our mantle cell lymphoma patients who underwent ASCT.

**Methods:** We retrospectively evaluated our 21 mantle cell NHL patients. The patients were followed after ASCT for relapse

**Results:** Patients were followed by a median time of 56.9 months (range, 6-170 months). The median age at diagnosis was 45 (range, 18-69), female to male ratio 5:16. The stages and MIPI scores at diagnosis were as follows: 5% stage I, 19% stage III, 76% stage IV; Low MIPI 29%, intermediate MIPI 48% and high MIPI 23%. First line treatments were R-CHOP for 6 cycles in 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, 7-48 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- IBEAM 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%) were 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 underwent autologous peripheral blood stem cell transplantation. The 3 year overall survival was 71%

**Summary/Conclusions:** ASCT is a part of initial treatment strategy in patients with mantle cell lymphoma however619 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 stereotyped verbal 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 ASD brain studies. Several 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 behavior.

**Results:** The first patient is an 11-year-old girl with ASD who was diagnosed 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 diagnosed 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 behaviors. According to CARS, prior to HSCT he had a score of 30 (Severe Symptoms of ASD Disorder), and he currently scores 24 (Minimal-to-No Symptoms of ASD). There is no treatment for ASD thus every effort to minimize the symptoms are valuable. In both cases, social interaction was significantly increased, and the aggressive behaviors decreased. Clinical cases have reported responses in autistic children receiving HSCT for acute lymphoblastic leukemia (ALL). Regardless of that, mantle cell lymphoma remains largely a relapsing/remitting disease. Today, novel agents may present a less intensive approach for achieving response.

**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 retrospectively evaluated 14 pediatric MDS patients who received HSCT at a single center. Median age at time of HSCT of the patients was 4 years and disease duration from diagnosis to transplantation ranged from 3 to 36 months with a median of 10 months. Five patients had primary and one had secondary MDS. Four patients had juvenile myelomonocytic leukemia (JMML) 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 6.8x10E8 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 (MDS), 5 from a matched unrelated donor (MUD) and 4 from haploididentical donor. Conditioning regimen consisted of busulfan/cyclophosphamide in MDS/MLD 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 TCRαβ/CD3 depletion.

**Results:** Graft failure occurred in three patients with JMML (n=1), secondary MDS (n=1) and MDR-AML (n=1). One patient with MDR-AML underwent second transplantation from another MUD one year after first transplant and died from GVHD. Ten patients are alive with a median follow-up of 19.5 months (range 3-61). All patients with primary MDS are alive and well. Four patients died from transplant-related toxicity (n=2) and relapse (n=2). For the entire group, estimated five-year relapse-free survival (RFS), event-free survival (EFS) and overall survival (OS) were 78.6%, 64.3% and 70.7%, respectively.

**Summary/Conclusions:** These data demonstrate that especially children with primary JMML can achieve encouraging OS and RFS following HSCT. Relapse remains the main cause of treatment failure in children with JMML given HSCT. All children with MDS should be referred for allogeneic HSCT soon after diagnosis.
PB2188

RELATIONSHIP BETWEEN URIC ACID LEVELS AND CARDINAL FINDINGS IN A LARGE COHORT OF B-THALASSEMA MAJOR: GENDER-RELATED DIFFERENCES

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Background: Iron overload, secondary to recurrent transfusions and ineffective erythropoiesis, induces oxidative stress in thalassemia (TM). Uric acid (UA), a major blood antioxidant, may act either as an antioxidant or pro-oxidant.

Aims: Our aim was to evaluate the role of UA in TM and its association with cardiac iron, dysfunction, fibrosis, and complications, and cardiovascular risk factors in a large cohort of TM patients of both sexes.

Methods: 397 TM patients (200 men, mean age 32±8 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) Network were considered. Multivariate and hierarchical cluster analysis were performed using the T2* technique. Atrial dimensions and biventricular function were quantified by cine images. Late gadolinium enhancement (LGE) images were acquired to detect myocardial fibrosis.

Results: As expected, UA resulted significantly higher in male respect to female TM patients (4.7±4.1 vs 3.8±4.0 mg/dL; P=0.0001). UA levels directly correlated with BMI (R=0.25, P=0.0033), 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±3.9 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* (β=5.7, P<0.001), and BMI (β=1.9, P=0.05) remained as independent determinants of UA in male TM patients.

Summary/Conclusions: UA levels correlate with factors related to metabolic dysfunction in TM patients of both sex, while a more strong correlation between UA and cardiac fibrosis was observed only in females, and a direct relationship between UA and T2* global heart only in males. The differences in male and female TM patients imply some gender-specific mechanisms, providing biochemical basis for the epidemiological differences between sexes.

PB2189

CHARACTERIZATION OF HEMORHEOLOGICAL ALTERATIONS IN THALASSEMIA MAJOR 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 circulatory disorders according to the viscoelastic behaviour.

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 to define a clearly identification of patients from the viscoelastic profile. Increased G’, G” and G* modula were observed in thalassemic patients demonstrating a reduction in deformability and impaired flow properties.

Summary/Conclusions: In this study a characterization of haemorheological alterations in thalassemia patients has been performed by a chemometric approach. The achieved results permit to consider the viscoelastic properties as promising predictive new indices of microvascular damage in β-thalassemia and to explain the increased incidence of vascular complications in these disorders.

PB2190

HEPATITIS E IN TRANSFUSION-DEPENDENT 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 an 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 18.5 years (2-60), 42% were males and 58% females. According to the patients’ blood transfusion history, the participants had been transfused with 47.376 blood units during the last 14 years, whereas during the last year the same patient population had been transfused with 3.384 blood units. The detection of HEV RNA was performed by Real-Time RT-PCR method (hepatitis2@ceerTools kit, Applied Biosystems AB), 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 implicated 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 the general population. Pro12Ala polymorphism may reduce the risk of cardiovascular complications. Consistently, Ala12 allele carriers were found to have lower corticoid 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 (21.53±5.44). 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 was 62.23±3.46% and 63.80±4.34 in cases and controls respectively. Pro12Ala polymorphism was present in 2 out of 30 (6.67%) β-TM patients with osteoporosis. One patient had heterozygous 12Ala polymorphism and the other had homozgyous 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 homozgyous patient). Ejection fraction was 70% in heterozygous patient and 68% in homozgyous patient. Only one male control (10%) has homozgyous 12Ala polymorphism (Table 1).

Table 1.

Summary/Conclusions: This study suggests that Pro12Ala polymorphism may have a cardioprotective effect in Egyptian thalassemic patients since we find the highest value of ejection fraction among the two positive cases. Further studies on a larger population of patients are still needed to confirm this finding.

PB2193

THALASSEMSMA 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, there is little data regarding the older population with TM and TI.

Aims: Our aim was to investigate characteristics of disease and patients and the Mann-Whitney test to compare between patients with TI and patients with TM.

Results: Between 2006 and 2016, 14 adult patients older than 35 years with TM (n=10) and TI (n=4) were followed and treated in our center. Median patients’ age was 37 (range, 35-51) years, with 66% males and 50% of Arab ethnicity. Most of the patients had at least high school education (85%), and 78% were employed. Thirteen patients (all TM patients and 3 out of the 4 TI patients) were treated regularly with blood transfusions. All patients received chelation treatment. Median hemoglobin (Hb) levels and mean corpuscular volume (MCV) levels were lower in patients with TI compared to TM (8.1 vs 10 g/dl, p=.002 and 72.4 vs 84 fl, p=.004, respectively). Median LDH levels and indirect bilirubin levels were higher in patients with TI compared to TM (603 vs 330 u/L, p=.004 and 2.02 vs 1.1 mg/dl, p=06, respectively) indicating increased hemolysis. All patients underwent splenectomy and had secondary thrombocytosis. All but two patients were treated with at least two different chelation modalities, either as single agent, including subcutaneous (SC) or intravenous (IV) deferioxamine (DFO), deferririne (DFP), or deferasirox (DFX), or as various combination therapy options. The median number of chelation treatment lines was 3. All patients treated with chelation suffered from at least one adverse event, necessitating temporary discontinuation and usually substitution of treatment. The median number of adverse events was 1.5 per patient. Nine patients (64.2%) had good compliance with current chelation therapy. Four patients with acute heart failure secondary to cardiac iron overload, and all four improved with intensified chelation treatment. Four TM patients (40%) were hypothyroid, half of them requiring thyroid hormone replacement therapy. All TM patients had hypogonadism. All females had amenorrhea and were treated with hormone replacement therapy, and none of them tried to conceive. Six of the seven male TM patients were treated with monthly testosterone injections, and three of them fathered children. All TM patients had osteoporosis, and three TI patients (75%) had metabolic bone disease. Figure 1 shows the relative rates of symptomatic cardiac iron overload and endocrine dysfunction in the cohort. Three patients (21.4%) had significant liver overload according to liver T2 MRI, necessitating chelation treatment intensification. None of the patients in our cohort underwent allogeneic hematopoietic stem cell transplantation and none developed secondary malignancy during follow-up.

Summary/Conclusions: Advances in the treatment of thalassemia patients have enabled the majority of these patients prolonged survival into adulthood. However, this has brought a new set of challenges for both patients and healthcare. This study delineates the challenges faced while treating adult patients with TI and TM in the new era.

PB2194

EVALUATION OF LIVER IRON CONCENTRATIONS IN CHILDREN WITH BETA THALASSEMIA INFECTED WITH HEPATITIS C VIRUS BEFORE AND AFTER SPIRULINA THERAPY BY MAGNETIC RESONANCE IMAGING

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Background: Magnetic resonance imaging (MRI) assessment of liver iron concentration (LIC) is necessary for quantitative staging of iron overload in children with β-Thalassemia. There is no enough evidence about the effect of spirulina therapy on LIC.

Aims: To assess LIC by MRI in multi-transfused β-thalassemic children infected with HCV before and after Spirulina Therapy.

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.

**PB2195**

**COMBINATION OF DEFERASIROX AND DEFEROXAMINE - A SUCCESSFUL CHELATION THERAPY IN β-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 thalassaemia (TDT) patients attending the Thalassemia Unit in a tertiary hospital in Athens, Greece.

**Methods:** 10 TDT patients were treated with a combination chelation therapy of DFX (30 ± 10mg/kg/d) and DFO (44±12mg/kg/d for 2-6 days/wk in 12hr or 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 deferoxamine (DFP) treatment and 3) adverse events recorded with increased doses of one of the chelating agents. The efficacy of the treatment was estimated through MRI accompanied with significant improvements in SF after spirulina therapy as compared to their pretreatment values in patients of the moderate to severe group.

**Results:** The efficacy of the treatment was estimated through MRI. 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.

**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 hemoglobinopathies. These involve the Variant II™ HPLC (BioRad), the Capillarys2 capillary electrophoresis (Sebia) and the most recently introduced HPLC, Premier Hb9210TM High Resolution HPLC of Trinty 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, HbD, 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-thalassemia carriers.

**Methods:** Hb variant separation using he Variant II™ HPLC (BioRad), the Capillarys2 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-thalassemia trait and haemoglobin (Hb) variants, including the molecular data of the beta-thalassemia mutations and Hb variants.

**Summary/Conclusions:** All three apparatus identified the common Hb variants and beta-thalassemia trait in carriers, homo- hetero- and compound het- erozygotes with the expected sensitivity and specificity. The Premier Hb9210TM HPLC was statistically superior in sensitivity when compared to the other devices determined by quantitation on the three different sites using the same samples and is suitable for the analysis of samples suspected of having hemoglobinopathy and the diagnosis of beta-thalassemia 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-He, which indicates the percentage of RBC with haemoglobin (Hb) content <17 pg, and %MicroR which indicates the percentage of RBC with mean cell volume <60 fl.

**Aims:** The aim of this study was to establish the reference range of our Laboratory for the parameters %HYPO-He & %MicroR, to investigate their values in haemoglobinopathies and their correlation, if any, with Hb A2 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 haemoglobinopathy heterozygotes (32 men, median age of 29 years & 57 women, median age of 30 years); and 317 patient samples for a variety of beta-thalassemia trait and haemoglobin (Hb) variants, including the molecular data of the beta-thalassemia mutations and Hb variants.

**Results:** The reference ranges of our Laboratory for the parameters %HYPO-He & %MicroR are 0.0 ~ 0.8% & 0.2 ~ 2.9%, respectively, and they are independent of gender and age (P=0.715, P=0.168 & P=0.0719, respectively). There was no statistically significant difference between the reference ranges of different age groups determined by one-way ANOVA for both parameters (all P > 0.0001). Heterozygous β-thalassaemia presents statistically significantly higher %HYPO-He values [11.0 (4.2-27.6)] as compared to groups A [1.0 (0.2-3.3)], C [1.9 (0.6-6.4)], D [0.6 (0.4-0.8)], while there was no statistically significant difference of %HYPO-He values between heterozygous Hb O-Arab and groups A and C [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

**Figure 1.**

**Summary/Conclusions:** Spirulina therapy may have favorable effects on lowering the values of LIC in children with β-Thalassemia infected with HCV.
Background: Diagnosing α-thalassaemia requires second line diagnostics involving DNA analysis. Multiplex ligation probe amplification® (MLPA®) is a molecular technique introduced as a diagnostic tool for α-thalassaemia. This semi-quantitative technique determines the relative copy number of up to 60 DNA sequences and is able to detect deletions and duplications in a DNA sample. A novel commercial tool, the α-Globin StripAssay®, aims to detect the most common α-thalassaemia deletions and point mutations. The test involves three steps: DNA isolation, PCR reaction and a hybridization step to test strip containing allele-specific oligonucleotide probes immobilised as an array of parallel lines.

Aims: Our objective was to evaluate the α-Globin StripAssay® as a useful alternative for MLPA® in second line α-thalassaemia diagnostics.

Methods: Eight samples, including 7 known deletions (α−SEA, α−THAI, α−MED, homozygous and heterozygous - α-3.7, heterozygous - α-4.2, (α)20.5) and 1 mutation (HB Constant Spring) were analysed using multiplex Gap-PCR (deletions) and Sanger sequencing (point mutation) at the Leiden University Medical Center. These samples were anonymised and analysed in duplicate by MLPA® and α-Globin StripAssay® at our center. A comparison of diagnostic performance, interpretation, turnaround time (TAT) and costs (reagent and labour) was done.

Results: There are no significant differences between the MLPA® and the α-Globin StripAssay® results and each identification corresponded to the result of the reference lab in Leiden. MLPA® however provided additional information about underlying polymorphisms. Interpretation of the α-Globin StripAssay® was easier and faster compared to MLPA®. The α-Globin StripAssay® proved to have a shorter TAT, but on the other hand, the costs for MLPA® were significantly less.

Summary/Conclusions: Despite its straightforward interpretation, shorter TAT and the possibility of detecting both (known) deletions and point mutations, the significantly higher costs of the α-Globin StripAssay® may hinder its routine use. Specialised laboratories are usually acquainted with the MLPA technique and in these settings the ability to detect both known and unknown deletions is a plus for research purposes.

PB2199

CARDIAC AND HEPATIC IRON ASSESSMENT OF YOUNG ADULTS WITH TRANSFUSION DEPENDENT THALASSEMIA: TIME TO THINK BEYOND FERRITIN

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Background: With the improvement in availability of blood transfusion practices and progress in chelation therapy, there is an increasing population of thalassaemic 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. For cardiac and liver iron assessment serum ferritin, ECG, 2D Echo, MUGA scan, Liver function test, Fibroscan (if indicated) and T2* MRI of Liver and heart was done. All patients who were diagnosed in childhood and referred to adult haematology unit at age ≥18 years and had received more than 20 blood transfusions were included in the study.

Results: A total of 53 patients (n=53) were analysed. The mean age was 23 years. Majority of patients (56%) were male. The average age at diagnosis and at first transfusion was 7 months & 11months respectively. The average years of PRBC transfusion was 23 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 hepatomegally 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 highest iron at the time of registration was 2919 ng/ml. LFT were deranged in 25% of patients. Evidence of cardiac dysfunction (ECG/MUGA) was present in 22% of patients. Iron overload in liver and heart as measured with T2* MRI was present in 56% & 28% respectively (Figure 1).

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.

PB2200

THALASSEMIAS IN MADRID: A PICTURE OF THE CURRENT SITUATION

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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. Aim: 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, unincentric, 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 Rumania. 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. Quleation 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 quelaion. Two out of 10 patients with Thal underwent
splenectomy. None of these patients had sepsis or meningitis. Three Thal patients underwent progenitor stem cell transplantations and two remain on complete chimerism in the present moment. Patients lost to follow-up summed up 14; 3 emigrated to other countries, 2 continue the monitor of their diseases in other centers or in adults units and 7 for unknown reasons. There was one death (3.22%) for a cause unrelated to his illness.

Table 1.

Summary/Conclusions: Early diagnosis derived from universal neonatal screening for sickle cell disease allows an effective health education and prompt therapy to other hemoglobinopathies, and a correct and thorough follow-up of these patients.

PB2201

PREVALENCE AND CAUSES OF CLOTTING TIMES PROLONGATION IN PATIENTS WITH TRANSFUSION DEPENDENT BETA THALASSEMIA

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Background: Thalassemia is traditionally known to be a thrombophilic, rather than hemorrhagic, disorder. In spite of this, prolongation of clotting times are often reported. Understanding if there is a real risk of bleeding, and what this risk can be associated to, is crucial, especially in relation to the frequent referral to surgery (e.g. for splenectomy, cholecystectomy). Hepatopathy due to iron overload or HCV infection has been addressed as a main cause of this finding, even though disorders in the clotting profile are often reported also in patients with no alterations of hepatic function. The impairment of factors XI and XII often reported has been hypothesized to be secondary to intravascular haemolysis or multiple transfusions (Caocci et al. Acta Haemtol 1978, Mcfadyen et al. Ann Hematol 2014), but no data are available to confirm this supposition.

Aims: To determine the prevalence of clotting disorders in a group of Transfusion dependent Thalassemia (TDT) patients and to assess the correlation with hepatopathy, degree of the hemolysis, transfusion frequency, erythroblastosis, iron chelation.

Methods: TDT patients followed at our center for whom clotting tests were available were included. From chart review data were collected regarding clotting times, demographics, disease history, comorbidities and anamnestic concomitant medications, iron chelation therapies, iron overload (serum ferritin, LIC, cardiac T2*), liver function tests, hemolysis parameters, hemocromocitometric values.

Patients on anticoagulation therapy were excluded.

Results: 22 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, genotyoe, transfusion interval, hemolysis degree, comorbidities, HCV infection included, iron overload, liver function, erythroblastosis and platelets levels, nor in history of thrombotic complications. No patients had history of hemorrhagic disease.

Pretransfusion Hb was lower in patients with prolonged clotting times (p=0.045); none of the patients in Group A was splenectomized (p=0.042).

Summary/Conclusions: In our population clotting disorders were not correlated with hepatic disease, nor hemolysis or transfusions. The mild correlation with lower Hb values and with the lacking splenectomy could be consistent with the known effect of low Ht on lab procedures for clotting tests. In relation to this observation in patients with altered coagulation tests the repetition of clotting test after blood transfusion could be advisable to overcome the low Hb effect.

PB2202

COMPOUND HETEROZYGOSITY 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 α-thalassaemia 3.7 kb deletion - the only α-thal and Hb Adana double heterozygosity cases diagnosed in subjects of Greek origin.

Methods: The first case concerns a 3 year old girl, born from parents referred for genetic counseling at the 11th week of a second gestation. The mother showed an Hb of 10.7 g/dl, MCV 80.7 fl, MCH 26.4 pg, Hb A 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 13.8 g/dl, MCV 8.5 X 109 /L, MCH 73.1 fl, MCH 23.5 pg, Hb A2 2.4% and Hb F 2.3%, while her ferritin levels were 228ng/ml and inclusion bodies were found. On clinical examination she was found to be of normal weight and height for her age, but presented with paleness, icteric sclera and mild splenomegaly. Genetic analysis revealed that the mother carried the α-thalassaemia 3.7 kb deletion defect. The father carried the rare non deletional Hb Adana. As suspected from the haematological aspects, their offspring was a compound heterozygote for Hb Adana variant in trans with a 3.7 kb α-thal deletion. The second case concerns an 17 year old boy, diagnosed with Hb Adana co-inheritance with the α-thalassaemia 3.7 kb deletion at the age of 8 years. At diagnosis, findings were compatible with a very mild phenotype and growth was not impaired. The boy retained a mild hypochromic microcytic anemia (Hb~10g/dl, MCV 71 fl, MCH 23 pg, RDW 18.6%, reticulo 5%), until age 11, at which age the treatment was decided due to marked splenomegaly and limited weight and height gain. For the following years he was transfused approximately once a month, necessitating chelation therapy. Weight, height and pubertal development were normal by the age of 15, but splenomegaly persisted. Splenectomy was decided and transfusion were stopped shortly afterwards. During the following months the boy retained an Hb of 9.5 g/dl, however, he complained of constant fatigue and impaired physical activity and asked to get back on a transfusion program.

Results: In both cases diagnosis was incidental highlighting the mild phenotype. However, the co inheritance of Hb Adana with the 3.7 kb α-thal deletion is rare, with only the presenting cases in Greece, and in a few other families in Turkey, Southeast Asia, Philippines and Albania. The clinical phenotype of the combination seems to be a mild disease with a non-transfusion-dependent thalassaemia intermedia phenotype. Nonetheless, clinical severity prediction is always a difficult issue and phenotypes may change overtime as demonstrated by the second case described above.

Summary/Conclusions: Long follow-up of such rare cases is necessary in order to gain as much information as possible, so as to offer the best management to the patients and the most accurate genetic counseling.

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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 x jugularis (Wessler model). The degree of thrombus formation was estimated on thrombus weight. Fibrinolytic activity and activity of tissue-plasminogen activator (t-PA) of blood plasma were measured by fibrin plate method. Anticoagulant activity (APTT-test) and ADP-induced platelet aggregation were detected by standard methods.

Results: Our experiments demonstrated that preliminary intranasal administration of PGPL (before formation of thrombus) leads to increase in APTT, fibrinolytic and t-PA activity on 18%, 62%, 35% accordingly from control rats. Besides, we observed the decrease of platelet aggregation. Also we indicated the reduction of thrombus weight in PGPL-treated rats on 68.5% comparatively with control rats. The thrombus weight after leucine treatment decreased on 30% compared with control rats. But administration of leucine did not change of haemostasis system parameters.

Summary/Conclusions: Thus administration of PGPL enhanced of anticoagulant, fibrinolytic and antiplatelet activity in rats blood plasma. PGPL pretreatment lead to prevention of experimental venous thrombus formation. Therefore, PGPL may be used as perspective anticoagulant and fibrinolytic agent with direct antithrombotic effect.

PB2204

TREATMENT AND OUTCOME OF THROMBOTIC MICROANGIOPATHY IN MALAYSIA

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Background: Thrombotic Thrombocytopenic Purpura (TTP) is a potentially lethal disease that there is still no promising cure in this era. The ADAMTS-13 activity in TTP was 4.36 times higher in patients with ADAMTS-13 activity >10% (OR: 4.36, 95% CL: 1.0961, 17.3714), indicating secondary TTP may have inferior treatment and outcome.

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 x jugularis (Wessler model). The degree of thrombus formation was estimated on thrombus weight. Fibrinolytic activity and activity of tissue-plasminogen activator (t-PA) of blood plasma were measured by fibrin plate method. Anticoagulant activity (APTT-test) and ADP-induced platelet aggregation were detected by standard methods.

Results: Our experiments demonstrated that preliminary intranasal administration of PGPL (before formation of thrombus) leads to increase in APTT, fibrinolytic and t-PA activity on 18%, 62%, 35% accordingly from control rats. Besides, we observed the decrease of platelet aggregation. Also we indicated the reduction of thrombus weight in PGPL-treated rats on 68.5% comparatively with control rats. The thrombus weight after leucine treatment decreased on 30% compared with control rats. But administration of leucine did not change of haemostasis system parameters.

Summary/Conclusions: Thus administration of PGPL enhanced of anticoagulant, fibrinolytic and antiplatelet activity in rats blood plasma. PGPL pretreatment lead to prevention of experimental venous thrombus formation. Therefore, PGPL may be used as perspective anticoagulant and fibrinolytic agent with direct antithrombotic effect.

Table 1.

<table>
<thead>
<tr>
<th>n</th>
<th>Gender (Age)</th>
<th>Anti-phospholipid antibodies</th>
<th>Anti-B2 (HbA1C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>Male (40)</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>50</td>
<td>Female (50)</td>
<td>25</td>
<td>75</td>
</tr>
</tbody>
</table>

Summary/Conclusions: Hematologic, renal and pulmonary involvement are the most common complications in patients with antiphospholipid antibodies positive. PGPL-treated patients had a better disease outcomes than primary TTP like congenital or acquired TTP. Besides, the complications of the disease were also evaluated which revealed 26.9% of renal failure and 52.2% of neurological deficit. Furthermore, 8.7% were complicated by Venous Thromboembolism, either provoked or spontaneous. The odds of relapse is 2.9 times higher given the ADAMTS-13 activity ≤10% to ADAMTS-13 activity >10%.
Background: Antiphospholipid antibodies (APLS) have been implicated in vascular (arterial, venous or both) thrombosis. Diabetes Mellitus (DM), as a disease entity has been associated with hyper-coagulable and pro-thrombotic states, with studies showing an increased procoagulant state and thrombotic events especially in poorly controlled Type 2 Diabetes Mellitus (T2DM). Aims: The aim of the study is to assess the APLS and HbA1c levels and evaluate the correlation between APLS levels and HbA1c in T2DM patients with diabetic vascular complications.

Methods: This was a cross-sectional study of subjects with T2DM attending the diabetic clinic of University of Nigeria Teaching Hospital. A total of two hundred and ten (210) subjects were recruited for this study. There were grouped into three (Arterial, venous or both, T2DM-uncomplicated T2DM and health control. Each had 70 subjects matched for sex and age. Lupus anticoagulant (LA) was assayed using DRVVT (technocline GmbH Austria) IgGβ2GPI-ACA was assayed using ELISA test kit (Genway Bio-tech San Diego USA), HbA1C was assayed using D10TM haemoglobin analyzer. Ethical clearance was obtained from the ethical committee UNTH.

Results: The prevalence of LA was 7.1%, 4.3% and 4.3% for complicated T2DM, uncomplicated and healthy control subjects respectively, while the prevalence of IgG-B2GPI ACA was 4.3% in all groups. The mean HbA1C were 8.2(1.5), 8.0 (1.7), 5.6 (0.3) for complicated, uncomplicated T2DM and control subjects respectively. ANOVA showed a significant difference in mean position HbA1C among complicated uncomplicated T2DM and healthy controls. Post hoc analysis showed this difference was between complicated T2DM and healthy controls (p=0.001, 95%, CI-3.0 to -2.1) and in uncomplicated T2DM and healthy control subjects (p=0.001, 95%, CI-2.8 to -2.0) there was a significant difference (p<0.05). The mean platelet aggregation was 88% for 12.5 μg/mL of fibrinogenase and by 80.5% for 12.5 μg/mL of fibrinogenase and by 80.5% for 12.5 μg/mL of fibrinogenase and by 80.5% for 12.5 μg/mL of fibrinogenase respectively. The mean time to platelet aggregation by hydrolyzing the Aα-chain of fibrinogen to prevent fibrinogen from combining with fibrinogen receptor on platelet membrane. In addition, fibrinogen degradation products may act as competitive inhibitors of platelet–fibrinogen aggregation and blood coagulation.

Summary/Conclusions: The study did not find any causal or other association between T2DM and the occurrence of APLS positivity, however, APLS may be simply an aggravating factor for vascular complications especially in poor controlled T2DM.

PB2207

VWF THR789AL AGENIC VARIANTS CORRELATE WITH DISEASE PHENOTYPE IN EGYPTIAN PATIENTS WITH ACUTE CORONARY SYNDROME

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Background: von Willebrand factor antigen level (vWF:Ag) was shown to contribute to the risk of cardiovascular disease. vWF THR789Ala single nucleotide polymorphism is thought to affect factor level and function. Aims: This study was designed to investigate the association of genetic variants at that position on the risk of acute coronary syndrome (ACS).

Methods: The study included 112 patients of ACS, 31 with unstable angina (UA) and 81 with myocardial infarction (MI) as well as 118 healthy controls. vWF:Ag level was measured by ELISA. The gene analysis was carried out by polymerase chain reaction using restriction fragment length polymorphism (RFLP-PCR) principles.

Results: vWF:Ag levels were significantly higher in MI (111.6±24.77 IU/dl) and UA (110.2±23.44 IU/ml) patients compared to healthy controls (71.1±13.72 IU/dl), p<0.001 for both groups. The majority of patients with UA (80.6%) were Ala789 homozygous, 6.5% were Thr789Ala heterozygous and 12.9% were Thr789 homozygous. Regarding the MI group, Ala789 genotype was present in 34.6%, Thr789Ala genotype was the predominant genotype and was seen in 48.1% of patients and Thr789 homozygous was present in 17.3% of patients. The genotype frequency in the control group was as follow: 47.4% were Ala789 homozygous, 33.1% were heterozygous and 19.5% were Thr789Ala heterozygous and 12.9% were Thr789 homozygous. The genotype distribution was significantly different among the 3 groups, p<0.001, and between the groups with UA and MI, p<0.001. Ala789 homozygous genotype was an independent risk factor for UA while the Thr789Ala genotype was shown as an independent risk factor of MI. Summary: vWF THR789Ala genetic variants are independent risk factors for DVT and has significant impact on the type of myocardial ischemia. It should be incorporated in a risk assessment model to identify individual patient risk and guide the management plan.
PB2210
HEREDITARY RISK FACTORS OF VENOUS THROMBOEMBOLISM IN YOUNG WOMEN TAKING ESTROGEN DRUGS
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Background: Estrogens are recognized as the most common risk factor of venous thromboembolism (VTE) in young women. The cumulative risk of VTE in patients taking estrogens is significantly increased in carriers of inherited thrombophilia. However, the known hereditary risk factors – mutations FV Leiden and FII G20210A could be detected in only 20-30% of patients with VTE.

Aims: To identify the most common hereditary risk factors of VTE in young women taking estrogen drugs.

Methods: We examined 133 young women with acute VTE (mean age 37.4 years; 16-45), who were genotyped by PCR-RFLP method for DNA polymorphism in 9 genes: FV Thr312Ala, FII -455AA, FII 20210A/G, FVIIa 46 C/T, FVIIa-A Val34Leu, PAI-1 -675 4G/5G, EPCR Ser219Gly, TPA 311bp Ins/Ins, PAI-1 -675 4G/5G, EPCR Ser219Gly, TPA 311bp Ins/Ins. We compared the distribution of studied genetic groups in three categories of patients with VTE: taking estrogens (n=30, group 1), with idiopathic VTE (n=42, group 2) or having other risk factors (n=61, group 3). Intergroup differences in genotype frequencies were assessed by Fisher's exact test. Odds ratios (OR), their 95% confidence intervals (CI) and p-values were calculated with SPSS software version 17.0 (SPSS Inc, Chicago, IL, USA).

Results: The frequencies of prothrombotic genotypes in groups 1, 2 and 3, respectively, were: FV 1691G/A – 20.0%, 21.4% and 13.1%; PAI-1 20210G/A – 10.0%, 9.8% and 7.1%; FII -455AA – 10.0%, 2.4% and 1.6%; FVIIa 46 C/T – 13.3%, 14.3% and 13.1%; PAI-1 -675 4G/5G – 16.7%, 28.6% and 31.1%; PAI-1 -675 4G/5G – 16.7%, 28.6% and 31.1%; PAI-1 -675 4G/5G – 23.0%; EPCR 219Gly – 16.7%, 19.0% and 23.0%; EPCR 219Gly – 3.3%, 7.1% and 0.0%; FXII 46 C/T – 13.3%, 0.0% and 9.8%; F XII-III A4 Leu/Leu – 3.3%, 21.4% and 9.8%. Significant differences between the groups have been detected only for the FXIII-A4 Leu/Leu variant, which was more frequently found in patients with idiopathic VTE than in the group of patients with estrogens (OR=6.5; 95% CI: 1.2-63.4; p=0.012) and women having other risk factors (OR=2.2; 95% CI: 0.8-7.6; p=0.05).

The frequency of FII -455AA genotype in young women with DVT developed after taking estrogen drugs was 4- and 6-fold higher, respectively, when compared to the group with idiopathic VTE and patients having other risk factors (10.0% vs 2.4%; OR=4.1, 95% CI: 0.02-2.2; p=0.16 and 10.0% vs 1.6%; OR=6.6, 95% CI: 0.7-67.0, p=0.067).

Summary/Conclusions: FV Leiden and FII 20210G/A mutations as well as the FII -455AA genotype are frequently seen in young women with DVT developed after taking estrogen drugs. Further studies are needed to clarify genetic risk factors contributing to VTE development in this group.

PB2211

Abstract withdrawn.

PB2212

Abstract withdrawn.

PB2213

KNOWLEDGE AND ATTITUDE OF MEDICAL DOCTORS ON ANTIICOAGULATION THERAPY IN TERTIARY HOSPITALS IN NIGERIA
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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 therapy in tertiary hospitals in Nigeria.

Methods: The present study is a multicentre survey of the use of anticoagulants among clinicians in South East Nigeria. A pretested questionnaire was administered to clinicians in six tertiary hospitals in the south-east of Nigeria. The following institutions participated in the survey: University of Nigeria Teaching Hospital Enugu, Federal Medical Centre, Abakiliki, Federal Medical Centre Umunhua, Abia State Teaching Hospital, Aba, Amaku Specialist Hospital, Awka and Nnamdi Azikiwe Teaching Hospital, Nnewi. The Likert scale which is in grades from one to five: 1 strongly disagree, 2 disagree, 3 neutral, 4 agree, 5 strongly disagree was used. To determine the agreement degree three levels were used (high, medium and low).

Results: There were 528 respondents. 378 of them were males (71.6%) and 150 were females (28.4%). 31.1% of the respondents were junior residents and the consultants represented only 20.6% of the respondents. Most of the respondents, 189 (35.8%) had less than 5 years clinical experience while the least, 36 respondents (6.7%) had between 16-20 years clinical experience. We observed that most respondents irrespective of their job grades didn’t know about Fondaparinux and the DOAC (except those in the specialist - registrar job grades) as the overall p=0.000.<0.05 and was significant. We also observed that responses that were divided on malignancy as an indication to use anticoagulation therapy/prophylaxis was clinically important had the highest mean of 4.60 and had a high degree of agreement. The statement “Should hospital inpatient with >3 days admission routinely receive anticoagulation?” had the lowest mean of 2.27 with a p-value of 0.015 had a low degree of agreement. The statement “Do you think anticoagulation therapy/prophylaxis is clinically important” had the lowest of their mean. All except one variable had p-values of <0.05. The statement “Should clinician be aware of the pharmacodynamic and pharmacokinetic changes of anticoagulants” had the lowest of their mean. All except one variable had p-values of <0.05. The statement “Do you think anticoagulation therapy/prophylaxis is clinically important” had the highest mean of 4.60 and had a high degree of agreement. The statement “Should hospital inpatient with >3 days admission routinely receive anticoagulation?” had the lowest mean of 2.27 with a p-value of 0.015 had a low degree of agreement. The statement “Should hospital inpatient with >3 days admission routinely receive anticoagulation?” had the lowest mean of 2.27 with a p-value of 0.015 had a low degree of agreement. The statement “Should hospital inpatient with >3 days admission routinely receive anticoagulation?” had the lowest mean of 2.27 with a p-value of 0.015 had a low degree of agreement.

Summary/Conclusions: There is a need to upscale knowledge attitude and practice of the use anticoagulation agents especially the NOACs through well-articulated CME educational activities. A limitation of this study is the relatively small number of study participants and some sub specialties that were not reflected in this study survey.

PB2221

INTERLEUKIN -10 GENE POLYMORPHISMS AND THE RISK OF UNPROVOKED DVT IN EGYPTIAN PATIENTS
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Background: Thrombosis is often multifactorial, caused by both genetic and acquired risk factors. The inflammatory process is linked to pathogenesis of venous thrombosis. Venous thrombosis is considered to be mediated by an imbalance in proinflammatory as compared with anti-inflammatory mediators. One of the important anti-inflammatory cytokine is interleukin-10 (IL-10) with important immunoregulatory functions. Primarily, IL-10 counterbalances the potentially harmful effects of tumor necrosis factor α (TNFα) and other proinflammatory mediator such as IL-1, IL-8, and IL-8 from monocytes/macrophages. Three important single nucleotide polymorphisms (SNPs) have been confirmed to predict IL-10 expression, including: 1082 A/G, 819 C/T, and 592 C/A. Studying the association between genetic polymorphisms of anti-inflammatory cytokines such as IL-10, and venous thrombosis may suggest using of polymorphisms as a predictive genetic marker of future VTE.

Aims: The objective of this study was to evaluate a possible association between IL-10-1082A/G, and -592C/A polymorphisms with DVT.

Methods: The study was conducted on 115 patients with symptomatic DVT proved by venous duplex ultrasound; divided into two cohorts: group A included...
60 patients with unprovoked DVT, and group B included 55 patients with pro-
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 pro-
provoked or non-provoked DVT. We also investigated the link between these poly-
morphisms and the recurrence of DVT and family history of DVT.

Results: In group A (IL-10 -1082AG) as well as group B (IL-10 -592C/A), no statistically
significant difference was found in the genotype distribution between the two
groups. No association was found between IL-10 gene polymorphisms and recur-
cence of DVT.

Background: Catastrophic antiphospholipid syndrome (CAPS) is a rare and
life-threatening event characterized by widespread intravascular thrombosis and
multi-organ dysfunction. It is a clinical condition of hyper-coagulability, 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
immuno-modulation. (B) Treatment of underlying infectious trigger. (C) Judi-
cious anticoagulation and treatment of the precipitating infec-
tions or positive family history.

Summary/Conclusions: 1. IL101082AG gene polymorphism is associated with
risk of unprovoked DVT, however it is not associated with either risk of recur-
rence or positive family history.

PB2215

CATASTROPHIC ANTI-PHOSPHOLIPID SYNDROME TRIGGERED BY
SEPSIS. A PROSPECTIVE CASE STUDY HIGHLIGHTING BIOLOGICAL
CONCEPTS AND MANAGEMENT STRATEGIES IN THIS COMPLEX AND
LIFE THREATENING DISEASE

M. Hua 1

1Haematology, Liverpool Hospital, Sydney, Australia

Background: Catastrophic antiphospholipid syndrome (CAPS) is a rare and
life-threatening event characterized by widespread intravascular thrombosis and
multi-organ dysfunction. It is a clinical condition of hyper-coagulability, 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: Describe the role of sepsis in triggering life threatening CAPS, and
highlight the management strategies used in this highly complex and fatal dis-
ease.

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
equivalents were lupus anticoagulant and anti-cardiolipin antibodies. Her haemostatic laboratory was normal. A previous case of CAPS was described with severe headaches. Subsequent investigations demonstrated multiple atrial
intracranial haemorrhages followed by concurrent extensive cerebral venous thrombosis. 2nd episode (2017): She presented with subdural haemorrhage, preceded by fevers and respiratory symptoms. She then de-
veloped pleuritic chest pain and dyspnoea after temporary cessation of antico-
agulation for 24 hours. Imaging confirmed multiple pulmonary emboli with
area of infarction. Respiratory symptoms worsened with progressive inter-
stitial ground glass changes on CT consistent with atypical pulmonary infection.
Shortly after low therapeutic anti-coagulation she developed acute abdominal
pain and hypertension. CT demonstrated significant bilateral renal and splenic
haemorrhages. Management Strategies: (A) Rapid reduction in APS patho-
genic auto-antibodies via plasma exchange, B cell depletion therapy and
immuno modulation. (B) Treatment of underlying infectious trigger. (C) Judi-
cious anticoagulation with anti-Xa monitoring and (D) long term hydroxy-
chloroquine therapy and statin therapy in accordance with the Helsinki Declara-
tion. 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. Activ-
ator (t-PA) activity in a healthy organism and the development of the experi-
mental MS.

Methods: Experiments 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. Activ-
ator 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-Gly and Pro-Arg-
Gly to healthy animals resulted a reduction of platelet aggregation by 23% and
52% respectively in comparison with PBS. 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.

Table 1.

<table>
<thead>
<tr>
<th>Haematological correlate</th>
<th>Tests performed</th>
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<tbody>
<tr>
<td>Lupus anticoagulant</td>
<td>Coagulation test</td>
</tr>
<tr>
<td>Sickle cell Disease</td>
<td>Blood count, CRP</td>
</tr>
<tr>
<td>Paroxysmal nocturnal haemoglobinuria (PNH)</td>
<td>Blood count, FBC, Haemoglobin</td>
</tr>
<tr>
<td>Leucopenia, Anaemia, Coagulation test</td>
<td>Full blood count</td>
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PB2216

HAEMATOLOGICAL CORRELATES OF ISCHEMIC STROKE AND TRANSIENT ISCHEMIC ATTACK: LESSONS LEARNED

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Background: Haematomal abnormalities are known to cause Ischemic Stroke or Transient Ischemic Attack (TIA). The identification of haematomal correlates plays an important role in management and secondary prevention.

Aims: The objective of this study was to describe haematomal correlates of stroke and their association between stroke profile. The haematomal correlates screened were Lupus Anticoagulant, Dysfibroglobinemia, Paroxysmal nocturnal haemoglobinuria (PNH), Sickle cell disease, Systemic Lupus Erythe-
matosus (SLE) and Myeloproliferative Neoplasms (MPN).

Methods: A cross sectional descriptive study was conducted in a sample of 152 stroke patients referred to haematology department of National Hospital of Sri Lanka for thrombophilia screening. Following tests were performed to assess each haematomal correlates (Table 1).

Table 1.

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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 antiplatelet and fibrinolytic effects of the blood. Thus, arginine-containing peptides could potentially be used as antithrombotic drugs that protect the organism from the blood coagulation and thrombus formation.

PB2218

THE PRINCIPAL COMPONENT ANALYSIS USING CALIBRATED AUTOMATED THROMBROGRAM PARAMETERS AS A POTENTIAL QUALITY CONTROL FOR MEASURING PROCOAGULANT ACTIVITIES OF IMMUNOGLOBULINS

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Background: The calibrated automated thrombogram (CAT) is a method to monitor the generation of thrombin. It can be described by four variables: lag time, peak thrombin, time to peak, and velocity index. Currently, due to thromboembolic event related risks of immunoglobulins, the CAT is widely used to quantify the thrombogenic potential associated with immunoglobulin manufacturing processes and products. However, there is currently no officially approved method for such assessments and even this results are highly variable in inter-laboratories comparison. In this study, to obtain a summary score, we applied the principal component analysis (PCA) for these four outcomes measured from CAT method. The PCA is a statistical procedure concerned with elucidating the covariance structure of a set of variables. In particular it allows us to identify the principal directions in which the data varies.

Aims: In this study, our interest is to apply PCA method in order to find appropriate dose related with CAT variables and to reduce variation of procoagulant values in Immunoglobulin products.

Methods: The CAT are measured in a 96 well plate fluorometer equipped with a 390/460 filter set and a dispenser. Usually experiments are carried out in triplicate. During the measurement, a dedicated software program, Thrombinscope compares the readings from the trigger wells and the calibrator wells, calculates thrombin concentration and displays the thrombin concentration in time. Outcomes from CAT were analyzed in the principal component analysis (PCA) which is a statistical procedure that allows us to summarize high dimensional data with a smaller number of representative variables that collectively explain most of the variability. Statistical analyses were performed with R 2.5.

Results: Four variables measured from CAT have different sistribution and too large variations. For example, the mean(sd) of each variable (lag time, peak thrombin, time to peak, and velocity index) are 24.86(8.01), 80.16(94.52), 31.28(9.78), 19.08(28.86), respectively. Therefore, to remedy such high variability among variables and to find a score, PCA method is applied. Then the dose values calculated based on the PCA scores have mean 0.393 and a much smaller variation (sd=0.583) (Table 1).

Table 1.

Summary/Conclusions: The PCA value showed a good agreement with four CAT outcomes and less variation. The PCA method could be used to monitor the process of immunoglobulin manufacturing.

PB2219

PRIMARY THROMBOPHILIA IN MEXICO XII: MISCARRIAGES ARE MORE FREQUENT IN PERSONS WITH THE STICKY PLATELET SYNDROME

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Background: The sticky platelet syndrome (SPS) is an inherited condition which leads to arterial and venous thrombosis. There is scant information about the association between the SPS and obstetric complications.

Aims: To assess the relationship of the SPS and fetal loss in a single institution.

Methods: The obstetric history of all the consecutive female patients prospectively studied along a 324 month period, in a single institute with a history of thrombosis and a clinical marker of primary thrombophilia was reviewed.

Results: Between 1989 and 2016, 268 consecutive patients with a clinical marker of primary thrombophilia and a history of arterial or venous thrombosis were studied; of these, 108 were female patients. Within this subset of thrombophilic female persons, 77 (71%) had been pregnant at some moment. Twenty eight of these 77 patients (37%) had had a spontaneous abortion and 24 out of these (86%) were found to have the SPS. On the other hand, in a subset of 73 female patients with the SPS who had been pregnant, 32% had miscarriages. These figures are significantly higher than the prevalence of abortions in the general population of pregnant women, with an odds ratio (OR)=13-14 (chi square=7.47; p=0.0063). Accordingly, the relative risk of having a miscarrage is 2.66 times higher in female patients with the SPS than in the general population (p=0.0014) (Figure 1).

Figure 1.

Summary/Conclusions: In México, female patients with the SPS experience significantly more spontaneous abortions than the general population. Since the treatment of the SPS is simple and effective and could in turn prevent adverse obstetric outcomes, its investigation in women studied because obstetric complications may be useful and deserves further research.

PB2220

CROSS-SECTIONAL ANALYSIS OF VENOUS THROMBOEMBOLISM IN YOUNG INDIAN MALES; NEW INSIGHTS INTO AN OLD PROBLEM

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Background: Venous thromboembolism (VTE) comprising of deep vein thrombosis (DVT) and pulmonary embolism (PE) is one of the major cardiovascular causes of death along with MI and stroke. Though earlier works have suggested that DVT is rarer in Asian population, recent studies have revealed that this might not be so. Most of the studies conducted in Asia in general and India specifically has been on hospitalized patients with minimal representation of young healthy individuals.

Aims: We aimed at studying the disease variables of VTE in young healthy males of Indian origin and compare the same with other Indian studies as well as the global statistics.

Methods: Hospital records of 176 Color Doppler Flow Index (CDFI) and/or Contrast Enhanced Computed Tomography (CECT) proven VTE patients being followed up in a tertiary care hospital was analyzed retrospectively to document cause (provoked/ unprovoked), venous systems involved, thrombophilia profile, duration of anti-coagulation and recurrence.

Results: Among the study population, 49.8% had a provoked VTE. 90.9% subjects had DVT, mostly of the lower limb. 15.3% had PE with DVT, 2.8% had PE alone and 6.2% had splanchnic vein thrombosis including portal vein thrombosis. In the subjects who had undergone thrombophilia profile, 41.9% had Protein C, 58.1% Protein S and 25.9% Antithrombin III deficiency. Lupus anti-coagulant screen was positive for 13% of the screened subjects. The average duration of anti-coagulation was 12 months with majority (98.2%) patients on Vitamin K antagonist. The recurrence rate in our study population was found to be 11.4% (Table 1).

Summary/Conclusions: Young Indian males have different disease variables
for VTE as compared to western population. The exact pathophysiology of such differences needs to be studied further to formulate strategies for effective screening and prevention.

Table 1.

PB2221

A PRELIMINARY STUDY ON THE EFFECTS OF AMPHIBIAN CRUDE SKIN SECRETIONS ON SOME PARAMETERS OF HEMOSTATIC SYSTEM

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Background: A lot of bio-chemical compounds from secretion of the amphibian skin glands with various biological activities have been isolated and characterized. Several recent studies indicate that amphibian skin secretions can be a source of molecules affecting the platelet activity. We are interested to look for other bioactive components of the amphibian skin which exhibit ability to influence on diverse parameters of hemostatic system.

Aims: We performed a preliminary study of the some effects of amphibian crude skin secretions on hemostasis.

Methods: Adult specimens (both sexes) of Bombina bombina, Bombina variegata, Bufo bufo, and B. 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 ultrafiltered using a membrane module (3 kDa cut-off) to remove large particulate and other suspended material. The filtrate was then used for experiments.

Results: The lyophilized Bufo 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. viridis also activated platelet aggregation but their effects were lower than B. bufo skin secretions. The skin secretions from all studied amphibian did not influence on PT and TT except B. viridis which prolonged TT by 40%. The values of APTT were significantly enhanced in 3.4 and 2.3 fold in comparison to control, respectively.

Summary/Conclusions: The obtained results indicate the prospects of the skin gland secretions to be used as 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.

PB2222

PLASMINOGEN-DEFICIENT PATIENTS

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1Istanbul University Cerrahpasa Medical Faculty, Pediatric Hematology-Oncology, 2Kanuni Sultan Süleyman Education and Research Hospital, Pediatric Hematology Oncology Dept., 3Istanbul University, Cerrahpasa Medical Faculty, Ophthalmology, Istanbul, 4Ankara University, Biotechnology Institute, Ankara, Turkey, 5University of Leipzig, Leipzig, Germany

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 local ophthalmic 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 Abruptio placentae 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 1C). When symptomaticatology 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.

876 | haematologica | 2017; 102(s2)
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 which 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 was identify 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 predominant was male in this group the symptomatology were related to physical efforts in a 60% (Paget-Shroetter Syndrome) whereas in female serie the predominant was thrombophic 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 cather related thrombosis and four cases thrombosis occurred in the conversion of subcutaneous anticoagulant. Two patients had a diagnosis of SLE. We had four cases of positive DD screening (both were marginally elevated, P <0.01 ). The risk of re-thrombosis was non significative but in the subanalysis of relapsing thrombotic event populations the risk of relapse increased proportionally in relation of thrombophilic 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 plants have an effect on the blood clotting system. It is known that there are heparin-like substances in some types of peony roots (Paeonia lactiflora, Paeonia suffruticosa), it proved that there is an anticoagulant activity in extracts from such roots.

Aims: The intention is to show the inhibitory effect of the extract of Paeonia lactiflora roots (EA) on processes fibrin and thrombus formation.

Methods: We used the standard coagulographic methods for determining anticoagulant activity by APTT test, antplatelet, total fibrinolytic activity (TFA), fibrin-depolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with thrombosis development and thromboembolia.

Results: It was shown that after administration of the indicated doses thromboplastin occurs hypercoagulability in blood plasma of animals (APTT decreased by 23% SFA - 15%, FDPA -12%; increased platelet aggregation by 18% compared to control animals not receiving thromboplastin). Normalization of blood clotting is installed in the experimental rats after application EP (recovery of platelet aggregation to 98%, APTT- to 100%, up to 95% SFA- FDPA and up to 67% compared with control). The high degree of FDPA indicates the ability of EP to obstruct the process of the formation of fibrins and thrombosis. Heparin components in EP interact with fibrin monomers which do not participate in their conversion to fibrin polymer. As a result, stable fibrin polymer or thrombus is not formed.

Summary/Conclusions: Consequently, the extract of Paeonia lactiflora roots containing heparinoid contributes to the restoration of coagulation properties in blood of animals in prothrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.

PB2225
THE INFLUENCE OF HEPARINOID FROM THE PEONY ROOTS ON THE THROMBUS DISSOLUTION
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Background: There is evidence that some of the peony roots (Paeonia lactiflora, Paeonia suffruticosa) contain heparinoid substances. It is believed that these substances have anticoagulant properties.

Aims: The study was aimed at investigating the influence of the extract of peony roots on the thrombus dissolution in animal models.

Methods: We used the standard coagulographic and biochemical methods for determining anticoagulant activity by APTT test, antplatelet, total fibrinolytic activity (TFA), fibrin-depolymerizing activity (FDPA). Experiments were carried out in accordance with ethical principles and documents recommended by the Declaration of Helsinki of the humane treatment of animals. We used an animal model with thrombosis development and thromboembolia.

Results: It was shown that after administration of the indicated doses thromboplastin occurs hypercoagulability in blood plasma of animals (APTT decreased by 23% SFA - 15%, FDPA -12%; increased platelet aggregation by 18% compared to control animals not receiving thromboplastin). Normalization of blood clotting is installed in the experimental rats after application EP (recovery of platelet aggregation to 98%, APTT- to 100%, up to 95% SFA- FDPA and up to 67% compared with control). The high degree of FDPA indicates the ability of EP to obstruct the process of the formation of fibrins and thrombosis. Heparin components in EP interact with fibrin monomers which do not participate in their conversion to fibrin polymer. As a result, stable fibrin polymer or thrombus is not formed.

Summary/Conclusions: Consequently, the extract of Paeonia lactiflora roots containing heparinoid contributes to the restoration of coagulation properties in blood of animals in prothrombotic condition and prevents thrombosis. In the initial stages of fibrin formation, it causes the thrombus dissolution.

PB2226
LOW MOLECULAR WEIGHT HEPARIN AND HIGH MOLECULAR WEIGHT HEPARIN: COMBINATION WITH ADRENORECEPTOR ANTAGONISTS AND PREVENTION OF THROMBUS FORMATION
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Background: Rethrombosis and thromboembolism are the most common side effects of thrombolytic therapy. One of the possible causes of thrombosis is the entering of thromboplastin in the blood stream. Marker of thromboplastin is an intrinsic membrane glycoprotein 5'-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 connected with the stimulation of the sympathetic nervous system. Besides, it is very important to study α-adrenoreceptor blocking agents effects on fibrinolytic and antiplatelet effects. The prevention of thrombosis complication is a very important field of pathophysiology and medical practices. Therefore, we studied effects of different α-adrenoreceptor antagonists and the influence of these substances combinations with various anticoagulant and fibrinolytic agents on the prevention of thrombosis.

Aims: The study of the influence of low molecular weight heparin (LMWH, 4.4 kD) and high molecular weight heparin (HMWH) and their combinations with different α-adrenoreceptor antagonists (AA) on experimental thrombosis preven-

Methods: Experiments were carry out on 50 white laboratory rats weighing 200-230 g according to the ethical principles of the Helsinki Declaration. Anticoagulant 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 digly-droergotoxin (DET – 1mg/kg), α–1–AA prazosin (PZ – 2mg/kg), LMWH or HMWH (40 USP/kg) were injected in v.jugularis. Saline was administered 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 timer of TF (arterio-venous shunt model). In blood plasma the activity of 5'-NT was detected. The processes was normalized with the decrease of blood pressure (on 40-50 mmHg). In this case the time of TF was prolonged in 4 time (LMWH) or 2 time (HMWH) comparatively with saline group 15 min after injection; in 4-5.5 time (DET+ LMWH or PZ+LMWH) and in 1-1.5 time (DET+ HMWH) or in 3-3.5 time (DET+HMWH or PZ+HMWH ) comparatively with saline group 180 min after injection.

Summary/Conclusions: Thus, we confirmed that LMWH (as one, as in combination with α-adrenoreceptor antagonists) has definite advantages over HMWH. Besides our results show that α-adrenoreceptor antagonists significantly improve antithrombotic effect of anticoagulant agents (LMWH and HMWH). Therefore the combination of LMWH with selective or 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 189 (35.8%) and those with 15–20 years of practice 46 (8.7%) are in the minority. Only 52 of the respondents (9.8%) claimed their institutions had an anticoagulation policy while 274 (51.9%) of them said there was no such policy and 168 (31.2%) do not know of any policy. Unfractionated heparin was the most frequently used (96.8%) and fondaparinux was the most infrequently used (42%). Most of the prescriptions were done by younger clinicians who are the highest in number. The consultants prescribed heparin and warfarin most, with the newer anticoagulants taking the rear position. Only 193 (36.6%) of the respondents routinely prescribed anticoagulation therapy when indicated. 412 (78%) of respondents believe the risk of anticoagulation outweighs the benefits while 439 (83.1%) identified cost is an important variable in prescribing anticoagulation agent. Anti-coagulation prophylaxis was the most frequently used for patients immobilized or bedridden (94.1%); malignancy and atrial fibrillation were the most infrequent reasons for using anticoagulants 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 >4.0×10⁹/L and PLT >20×10⁹/L.

Results: 25 patients were included in the study of which 23 were brought into statistic. 13 patients were in experimental group and 10 in control group. There were no difference in age, gender and dose intensity of chemotherapy between the two groups (P>0.05). The average recovery time of the blood neutrophil granulocyte >0.5×10⁹/L in experimental group and control group were respectively (8.53± 3.26) days versus (13.12± 5.76) days (P<0.05) and that of PLT >20×10⁹/L was respectively (9.24± 3.88 ) days versus (11.62± 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 to calculate for all various subspecialties including Surgery, Internal Medicine, Pediatrics and Obstetrics and Gynaecology. All procedures related to hospital transfusion practice were reviewed and re-evaluated to address gaps. Policy of maximum surgical blood ordering (MSBO) was implemented based upon both results of audits and by discussion and agreement between medical teams. Focused training and education has been followed to increase the awareness of the health care workers. Plus monitoring of C:T ratio on monthly basis, blood bank team had arranged meetings with the departments that were over-ordering cross-matches to explain that group & save test is a safe, effective and financially beneficial strategy. Communicating with the physicians had been the most challenging aspect of implementing the policy changes. Regular audits had been conducted to measure the compliance and effectiveness of the blood management practice.

Results: Compared to the international guidelines, C:T ratios in 2010 was beyond the acceptable target and ranged between 2.5 to 3.2 highlighting the over-ordered cross-matched blood in certain sub-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.
SAFETY AND EFFICACY OF A PROTHROMBIN COMPLEX CONCENTRATE PB2230 IN VKA REVERSAL AND OFF-LABEL INDICATIONS

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Background: Prothrombin complex concentrates (PCC) are highly purified mixtures of plasma coagulation factors that contain vitamin K dependent and anticoagulation factors, they are approved for urgent reversal of vitamin K antagonists (VKA). Massive bleeding-associated coagulopathy guidelines include PCC in their management, although as an off-label indication.

Aims: The aim of the present work is to evaluate safety and efficacy of PCC in a case series of VKA reversal and refractory coagulopathy associated with major bleeding.

Methods: Retrospective review of cases treated with a four-factor PCC between January 2010 to January 2016 in two tertiary University Hospitals.

Results: 328 patients were included (47.25% male), median age 78 years (range 19-102). PCC was used in the following cases: 1) 66.76% in VKA reversal (181 patients due to hemorrhage and 33 prior to emergent surgery), mean dose of PCC 1333.51 IU; 2) 30.54% in refractory coagulopathy in major bleeding (30 patients due to massive bleeding protocol activation, 43 patients in hepatic coagulopathy and 25 patients in bleeding not related with any of previous reasons) a mean dose of PCC 1681.63 IU was used. Safety endpoint: Two infusion reactions were reported potentially related to PCC use, they were not specified as anaphylaxia or as pulmonary edema, and 8 thrombotic episodes were observed (2.4%): 5 pulmonary embolism, 2 deep venous thrombosis and 1 portal thrombosis, 75% of the events appear in the group of VKA reversal. Efficacy endpoint: VKA reversal in bleeding patients was effective in 97% of them, 76.5% with complete reversal of INR value (INR<1.5), 34.25% of patients required fresh frozen plasma. Thrombocytopenia associated with major bleeding was 31.6%, having a worse outcome (40% rate of death) those who suffer a massive bleeding coagulopathy, all death related with absence of bleeding control. A global INR correction happen in 76.7% of patients, complete correction in 40.7%. 63.26% received previous to PCC fresh frozen plasma. Invasive hemostatic procedures were required in 20% of the whole series.

Summary/Conclusions: A four-factor prothrombin complex was safe and effective as adjuvant treatment in refractory coagulopathy due to major bleeding as well as for the emergent reversal of VKA.

TRACEABILITY OF RED BLOOD CELLS IN A HOSPITAL TRANSFUSION LABORATORY

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Background: According to European legislation (2002/98/EC, 2005/61/EC) as a requirement of hemovigilance system traceability (confirmation of final destination of blood components in hospitals) information should be kept for 30 years, improving the quality and safety of the transfusion process. Various methods are available from simple paper-based procedures to full electronic blood tracking systems. The ideal goal is to trace the final fate of 100% of the red blood cell (RBC) units, from donor to recipient and vice versa.

Aims: To check the ability to trace each individual unit from donor to recipient or disposal in our hospital.

Methods: To ensure compliance, the minimum traceability data set for retention is a mix of 1) Wards’ paper files (file of transfusions and/or patient records: 14/2 wards respectively), 2) HTL electronic records and paper records. The transfusion practitioner is responsible for the collection and maintenance of traceability data.

Results: During the year 2016, the number of RBC units transfused in our hospital was 2128. The traceability status of the transfused units is shown in the Table 1.

Summary/Conclusions: Although we are satisfied that the results represent a reasonably accurate working model of the current situation, the trail of a unit is less reliable after blood has left the HTL. 1. Patients’ notes to provide traceability are not totally reliable. It is apparent that the ward staff plays a key-role part in the chain and this highlights the need for them to receive training to emphasize the importance of their contribution to hospital compliance. 2. The indications are that the essential requirements on traceability are not fully met by the current laboratory computer system. A configuration is needed to produce a report which lists components which have been assigned for use but do not have an entry for return to stock or final fate. Ongoing problems will be referred to the Hospital Transfusion Committee. 3. For the longer term ultimately only effective IT system in both wards and HTL can ensure total traceability and we recommend the inclusion of electronic tracking system in the National Blood Donor Registry Programme (EMA)
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).

PB2235

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 native, homogeneous selection of peculiar haematologic characteristics, such as the absence of high frequency red cell antigens (defining a rare blood) that cannot be found in donors of European descent so that many SCD transfused patients experience the fearful occurrence of red cell alloimmunization. For these reasons haematologists are expecting to access to Rare Blood Banks in order to assure a full match between donor and recipient’s blood, that may be obtained from donors sharing the same ethnicity. Unfortunately SSA donor recruitment is counteracted by the widespread diffusion of infections contracted before migration: one of these is malaria. In SSA malaria may occur subclinically and is characterized by a slow antibody clearance. This peculiar condition, the so-called semi-immunity, has been induced by a strong genetic pressure, and is a kind of co-evolutionary process characterized by the co-existence and persistence of small entity of Plasmodium genome with relative antibodies. Molecular techniques are unreliable to detect a small number of Plasmodia, which may otherwise be sufficient to induce a transfusion transmitted malaria (TTM). The serologic assessment, despite the low specificity, remains the most sensitive and reliable method to detect the semi-immune status in blood donors (1).

Aims: The aim of this study was to assess the prevalence of malaria immunity in a cohort of healthy SSA citizens.

Methods: Since 2010 in our department of Haematology and Transfusion Medicine we recruited 184 SSA citizens, in good health, who agreed to underwent clinical and laboratory investigations to become a blood donor. All of them were born in SSA Africa and lived there for at least the first 5 years of life. 70% of subjects didn’t recognize any previous malaria fever. The last travel/stay in African countries (t=3 yrs), and 39% of returning people had received prophylaxis. Malaria serology was determined by a commercial enzyme immunoassay kit (Malaria EIA Ab, BioRad).

Results: Overall 75% of persons were positive for malaria antibodies. Serologic positivity was found in 75% of persons no more exposed in 5 recent years and even in 83% (19/23) persons settled in Italy since 10-20 years. Serologic positivity was present in 100% of people from Benin, 85% from Burkina Faso, 78% from Ivory Coast and Cameroon, 63% from Senegal. We followed antibody concentration in 50 persons (136 assays), and we observed a slightly negative trend that, in most cases, was followed by a prolonged phase of low antibody levels, 4/50 became negative after three years.

Summary/Conclusions: The identification of malaria antibodies is essential in SSA native donors and, by far, irreproducible in order to avoid the risk of TTM. Until pathogen inactivation techniques will become available, we have a very low expectation to introduce SSA blood in Blood Bank inventories. Haematologists have to be prepared years for an forthcoming SSA second generation that will allow to fully match the entire SSAD patient community.

REFERENCE


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 microg/l), evidence of iron overload or received over 20 units of red blood cell transfusions (RBCT). Deferasirox showedefficacy and safety in maintaining or reducing body iron (assessed by liveriron concentration or serum ferritin).Iron chelation therapy was associated with hematopoiesis improvement in transfusion-dependent patients and interruption of Deferasirox treatment of transfusions dependent myelodysplastic patients produced loss of erythroid response. Aims: Aim of the study: to assess the results of Deferasirox efficacy, side effects and to study if the number of RBCT decreased after starting Deferasirox.

Methods: We have done a retrospective, transversal study including all the adult politransfused patients treated with Deferasirox in three counties Haematology Departments of North-West Romanian hospitals. Criteria of Deferasirox treatment: over 20 RBCT, serum ferritin level over 1000 microg/l. We created a data collection sheet including: demographics, information on patients’ disease, serum ferritin level at start of and during treatment, Deferasirox dose, data about dose modification, adverse effects of Deferasirox and their management, reasons for treatment discontinuation, evaluation of comorbidities that could increase serum ferritin level, number of RBCT before and after starting the treatment.

Results: We included 40 politransfused patients treated with Deferasirox, age average 63. The diagnosis included meiolodysplastic syndromes (most of patients), thalassemia, other anemias. Myelodysplastic patients were treated with low dose chemotherapy, epigenetic treatment, RBCT and Deferasirox. Before transfusions patients were transfused. The baseline value of ferritin was between 1075 - 1617 microg/l. Deferasirox dose: 20-30 mg/kg. There was a significant reduction in serum ferritin from baseline for all the patients. Ferritine median at start, 3631 microg/l decreases at 1537 microg/l after 6 months of treatment and at 894 microg/l after 12 months of treatment. There were 8 patients that had a rapid decrease of ferritin, but during infectious episodes the ferritine increases for a short period of time. Digestive adverse events appeared in three cases (two cases of diarrhea and one case of digestive hemorrhagic episode). In all these cases the treatment was temporarily discontinued. In three cases, treatment was stopped because low ferritin level (under 500 microg/l). RBCT were administered before (mean 2.43 units/month) and after starting Deferasirox (mean 1.39 units/month), the difference is statistically significant (Student Test, t(39)=6.98, p<0.001). After starting Deferasirox treatment mean number of RBCT decreased, mean of differences (95% CI) was 1.04. We analyzed the group of 23 patients treated with Deferasirox less than 12 months, and the patients treated more than 12 months, 15 patients. In both groups the difference of RBCT means (before and after the start of the treatment) are statistically significant (for the patients treated less than 12 months: Student Test, t(23)=8.12, p<0.001 and for the patient treated more than 12 months: Student test, t(15)=3.03, p<0.008).

Summary/Conclusions: Analyzing our group of 40 patients, Deferasirox proves to be effective and safe. Adverse effects that determined a temporary stop of the treatment were mild/medium short time digestive reactions. The number of red blood cell transfusion significantly decreased after starting Deferasirox treatment.

PB2235

LIBERAL VS RESTRICTIVE COMPARATIVE TRANSFUSIONAL STUDY IN ONCOLOGICAL POPULATION

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Background: Allogeneic transfusion therapy is perhaps one of the most widely used treatments without good evidence support, despite many years of application in clinical practice. This, coupled with blood shortages, the impossibility of allogeneic zero risk, the lack of evidence that transfusion may increase consumption or decrease tissue oxygen debt and the existence of an association with an increase in morbidity and mortality have favoured that we join efforts towards its optimal use.

Aims: Optimal use in our adult oncological population and evidence that restrictive transfusion (TR, Hb 7-9 g/dl) is not greater or lowero the liberal transfusion (TL, Hb 8-10 g/dl), keeping hemorrhagic in safe levels for the patient. Methods: A research was performed from October 1st, 2015 through December 31st, 2016. We analyzed the proportion of patients receiving packed red cells (CH) and the number of units transfused as well as post-transfusion control in order to describe the outcome of the CH versus TL strategies in the cancer population under the study.

Results: See Table 1.

Summary/Conclusions: The results obtained in our series of 311 cancer
patients indicate that the restrictive strategy has been equally effective and probably superior to the liberal one maintaining Hb at a safe level in each patient, as well as quality of life and comfort in a subgroup with advanced and terminal cancer.

### Table 1.

<table>
<thead>
<tr>
<th>Transfusion Therapy</th>
<th>Patients (N)</th>
<th>Hb Pre (g/dL)</th>
<th>Hb Post (g/dL)</th>
<th>yield CH (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT</td>
<td>192</td>
<td>8.1</td>
<td>9.1</td>
<td>1.0</td>
</tr>
<tr>
<td>LT</td>
<td>97</td>
<td>7.4</td>
<td>9.4</td>
<td>1.0</td>
</tr>
<tr>
<td>PWC</td>
<td>33</td>
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<td>-</td>
</tr>
<tr>
<td>TPF</td>
<td>311</td>
<td>8.2</td>
<td>9.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Hb Pre: Pre-transfusion haemoglobin; Hb Post: Post-transfusion haemoglobin; PWC: Patients without post transfusion Hb level; TPF: Total Patients Transfused; X: half haemoglobin.

**PB2236**

**HIGH RISK OF HBV INFECTION IN VACCINATED POLYTRANSFUSED CHILDREN**

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**Background:** Children receiving chemotherapy for neoplastic diseases are still susceptible to Hepatitis B virus (HBV) infection despite the national HBV vaccination program coverage for all infants since 1992. This study aimed to analyze immunity against HBV and occurrence of HBV breakthrough infections in polytransfused children who had been vaccinated during infancy.

**Methods:** The study included 89 children with hematological disorders and malignancies, who were categorized into group (A): 37 receiving chemotherapy (M:F 20:17; mean age: 7.7±4.0) and group (B): 52 polytransfused children (M:F 31:21; mean age: 7.6±3.2). A matched healthy control group (n=162) was also included. All patients and controls had reached their primary vaccination against HBV in infancy. Quantitative anti-HBs were tested for patients and controls. Patients’ sera were tested for HBsAg, anti-HBC, and HBV-DNA (nested PCR for full virus genome & c-region).

**Results:** Levels of anti-HBs between 10-100 IU/L and ≥100 IU/L were found among 13.5% and 21.6% [group (A)] and 32.1% and 10.5% of controls respectively. There was a significant difference in HBsAb levels between patients receiving chemotherapy (group A) and both groups B patients (p<0.008) and controls (p=0.032). However, no difference was found between polytransfused children (group [B]) and controls. HBsAg was positive in 21 (67.7%) children under chemotherapy [group (A)] compared to 10 (32.2%) polytransfused children [group (B)] (p=0.0005). Overall, 49 patients (55%) were HBV-DNA positive; 44 c-region positive, 7 s-region positive. The positivity for both c and s-regions and one positive a,c and x-regions. Of these, 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 [group (B)] (p=0.157), regarding the rate of HBV DNA. Anti-HBs ≥10 IU/L co-existed in 38.7% (12/31) of HBsAg positive patients and 49% (24/49) of HBV-DNA positive patients.

**Summary/Conclusions:** Children with neoplastic diseases vaccinated during infancy were at a high risk for HBV infection. The effect of immunosuppression on the HBV protective level favored overt HBV infection in children receiving chemotherapy. The co-existence of anti-HBs with HBsAg and/or HBV-DNA demonstrated a possible residual transfusion-transmission risk with mutant HBV strains.

**PB2237**

**THE ISOHEMAGGLUTININ TITERS OF BLOOD BANK DONORS: THE EXPERIENCE OF ISTANBUL FACULTY OF MEDICINE**

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**Background:** Isohemagglutinins that develop against ABO blood group antigens are very important in transfusion and transplantation medicine. Today, 30-40% of allogeneic stem cell 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 titters according to the decades and gender. Also we examined the possibility of determining the isohemagglutinin cut off value in Turkish society.

**Methods:** One thousand five voluntary blood donors (48 female, 957 male), randomly chosen from the donors, providing the criteria to be a standard blood donor in Blood Center Department, Istanbul Faculty of Medicine were studied. This study was approved by the Ethics Committee of Istanbul Medical Faculty. In the donor population group; blood group A (%40) was the most common and blood group AB was the rarest blood group. 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 volun- tary 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 0 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 titer in blood group O between males and females(p>0.05). However Anti-B IgG and IgM antibody titers were higher in females than males in donors group O respectively p=0.017 (p<0.05) and p= 0.001 (p<0.01) (Figure 1 A,B).

**Table 1.**

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Titer</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1:128</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>1:256</td>
<td>20</td>
</tr>
<tr>
<td>O</td>
<td>1:512</td>
<td>30</td>
</tr>
</tbody>
</table>

**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 therapeutical approaches for risk analysis in fetal examination during pregnancy, perinatal complications causing fetomaternal hemorrhage after pregnancy or during birth and lastly autismous 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 titers of individuals. Population specific isohemagglutinin titer values play a key role in blood donation policy of patients undergoing hematopoietic stem cell transplantation. Consequently; we predict that Turkish community-specific isohemagglutinin cut off titer values can be identified and we will hope our knowledge on this issue in the future with the increase of research is going to increase further.

**PB2238**

**THE NEW METHOD OF PURIFICATION FACTOR COAGULATION VIII N. Shurko1,*, T. Danysh1, V. Novak2

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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.

**Methods:** We made a retrospective, descriptive analysis of ten cases diagnosed of primary thrombotic microangiopathy (TTP n=5; typical HUS n=3; atypical HUS n=2) over the last eight years, 70% of which were women with an average age between 40-60 years. Only three cases had previous records of autoimmune diseases (MTCRD, RA and HIV), all of which would eventually develop TTP. We requested ADAMTS13 levels on all cases, they were low (<5-10%) only in those patients diagnosed with TTP, and on the other hand confirming the positivity for Shiga toxin in those patients who eventually developed typical HUS.

**Results:** Regardless of the diagnosis, 10-12 plasma exchanges were performed to improve the biological parameters of hemolysis, requiring the placement of a central catheter, most commonly at the right jugular vein (70%) due to the lower risk of thrombotic and infectious complications. Although renal involvement is frequent in HUS, only two of the patients required dialysis without recovery of baseline renal function. Is to be noted the part played by the approval in 2011 of eculizumab and how patients eight years ago suffered a torpid course, requiring a greater number of plasmapheresis and the side effects this carries. However, we still do not know its repercussions out of indication. In our study, it was used in a patient with a diagnosis of HUS associated with an infection with good evolution, although perhaps this result is due only to the natural evolution of the disease. Another controversial point is the use of antibiotics, which are known to worsen the clinical course of these processes, but because of a concomitant infection or new positive determination of the Shiga toxin, as occurred with two of our patients diagnosed of HUS had to be used. Finally, 30% of the patients have relapsed after the first episode with a primary diagnosis of a TTP.

**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:

- The need to request levels of ADAMTS13 in patients diagnosed with TTP or to repeat the determination of Shiga toxin in patients with typical HUS as part of the disease follow-up and to try to prevent possible relapses.
- The use of eculizumab out of indication in typical HUS and whether the improvement in the picture is due to the drug or by natural evolution of the disease. Their real benefit using plasmapheresis in patients diagnosed with typical HUS.

**Aims:** To review our experience in the management of the primary TMA and to raise a series of questions that perhaps could improve the understanding of these pathologies.

**Background:** The real benefit of using plasmapheresis in patients diagnosed with typical HUS is of great concern.

**Methods:** To request levels of ADAMTS13 in patients diagnosed with TTP or to repeat the determination of Shiga toxin in patients with typical HUS as part of the disease follow-up and to try to prevent possible relapses.

**Summary/Conclusions:** The results of the study are helpful to identify and understand the complication of adverse donor reactions though the incidence of reactions of blood donation among blood donors in the tertiary care hospital in 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 donors in different locations including outdoor and in-house blood donation drive.

**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 hemotoma; 6(7.62%) while 16 (15.24%) were severe adverse reactions similarly, anaphylaxis; 11(10.49%), loss of consciousness; 9(8.58%) and convulsive syncope;2(1.90%). Markers of the adverse donor reaction were age, sex, pulse, weight, blood pressure and donation status. Age and first time status were related with significantly higher risk of adverse reaction with 18-23 years old at higher risk compared to 24-55 years old. First time donors were at higher risk compared to repeated volunteer donors.

**Summary/Conclusions:** The results of the study are helpful to identify and understand the complication of adverse donor reactions though the incidence of reactions in the blood donors is lower than in other studies. Donor age and donation status were strong possibilities of complications.
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Late Breaking Oral Session

LB2600

This abstract is part of the Presidential Symposium

NOVEL SMALL MOLECULE INHIBITORS CO-TARGETING CKIA AND P-TEFB DISRUPT SUPER-ENHANCERS AND ERADICATE ACUTE MYELOID LEUKEMIA IN A MOUSE MODEL

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Background: Whereas p53 is mostly non-mutated in AML, various oncogenic pathways, frequently through enhancing the activity of its major antagonist Mdm2, suppress its activity. We have previously showed that genetic ablation of CKIα robustly activates p53 (doi:10.1038/nature09673). However, with no selective CKIα inhibitors for in vivo use, the therapeutic value of CKIα inhibition in hematological malignancies cannot be validated.

Aims: To develop small molecule CKIα inhibitors and assess their effect in mouse models of human leukemia.

Methods: CKIα inhibitors were identified via cell-based screening based on p53 activation. We focused on a small class of pyrazole-pyrimidine scaffolds, which through extensive medicinal chemistry yielded derivatives with high affinity binding, validated by crystallography studies, potent CKIα inhibitory activity and a good pharmacokinetic profile. Anti-leukemic activity was assessed by oral treatment in mouse models of AML. MLL-AF9 and Bcr-Abl Blast Crisis Results: We first demonstrated the inhibitors’ anti-leukemic effect by single oral dose treatment, robustly inducing p53 activation and blast cell cytoreduction (Figure 1).

Figure 1.

These inhibitors distinguished leukemic from normal hematopoietic stem cells: they did not affect normal hematopoietic CFUs, but eliminated leukemic CFUs at an IC50 <9nM. We tested the long-term oral therapeutic effects of the inhibitors in MLL-AF9 leukemic mice. Whereas all vehicle-treated mice succumbed to the disease within a month, 40-50% of inhibitor-treated mice survived with no signs of disease up to 5 months’ observation, nor had the surviving mice any sequela of long-term treatment; all had normal blood counts and normal organ morphology and histology. Long-term leukemia control with possible cure, attesting to eradication of LSCs and preservation of normal 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 profiled the kinome and expressed genes of both + cases and those with CCND2 overexpression. The male/female ratio was 2.5:1 and median age at diagnosis 66 years.

Results: Most cyclin D1−MCL (49/51, 96%) showed overexpression of other G1 cyclins: CCND2 in 33/35 (94%), CCND3 in 12/51 (24%), and moderate overexpression of both CCNE1 and CCNE2 in 38/51 (75%), respectively. 7 cyclin D1−MCL were detected by FISH in 25/33 cases (76%) with CCND2 overexpression, but the remaining CCND2+ cases and those with CCND3 overexpression did not show CCND2, CCND3 and Ig rearrangements using currently used break-apart probes. Interestingly, by mate-pair whole-genome and whole-exome sequencing analyses we discovered crypic insertions of Ig light chain regions including the enhancer regulatory elements (2 IGK and 1 IGL) near CCND3 gene in the three cases with cyclin D3 overexpression. These rearrangements were confirmed by Sanger sequencing and FISH with specifically designed probes to recognize the rearranged IG regions. Additionally, using the same approach we identified 6 additional cases with crypic IGK-CCND3, as well as 3 cases with IGK-CCND2 juxtaposition in tumors with high levels of CCND3 and CCND2, respectively. Taken together, 74% and 18% cases corresponded to cyclin D2+ and cyclin D3+ MCL, respectively, whereas 6% showed overexpression of CCNE1 and CCNE2 without any Ig rearrangements. The whole-genome data with CCNE1 and CCNE2 overexpression identified 29 somatic protein-coding mutations, 42 complex structural variants and 24 copy number alterations (including CDKN2A and RB1 homozygous deletions) but non rearrangements involving any of the Ig genes or cyclins. The global genomic profile of 47 cyclin D1−MCL was highly complex with higher complexity, as compared to the conventional SOX11 MCL, with significantly more gains at 7p and 18q in the cyclin D1+ MCL. Moreover, 32% cases had chromothripsis at least in one chromosome.

Summary/Conclusions: We have identified a novel Ig light chain locus-associated rearrangement, consisting of cryptic insertion of Ig enhancer near CCND3 gene juxtaposition. Similar rearrangements were also observed in other cases of cryptic IGK enhancer region into CCND2 gene. Both aberrations were unidentifiable by cytogenetics and FISH break-apart approaches. Overall, 65/66 (98%) MCL had G1 cyclin overexpression. The detection of these rearrangements with cuss...
tom FISH probes or that of high levels of CNCD2 or CNCD3 by qPCR, together with SOX11 expression, helped to identify cyclin D1+ MCL and provide insights on the pathogenesis of this rare subgroup.

**LB2602**

**ARNT/HIF-1BETA LINKS POOR CLINICAL OUTCOME TO MICROENVIRONMENTAL HYPOXIA AND HIGH-RISK 1Q GAIN IN MULTIPLE MYELOMA**

F. Jin1, X. Liu1, C. Wu1, P. Yang1, X. Yu1, X. Wang2, L. Ye1, Y. Sun1, J. Sun1, S. Gao1, Y. Dai1

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**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-1B (HIF-1ß), a gene located in the 1q21 region, in 1q MM and hypoxic microenvironment.

**Methods:** The relationship between 1q or HIF-1ß 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-1ß and other 1q21 genes in 1q+ MM microarray database UAMS “Multiple Myeloma DataBase” (University of Arkansas) and whether CD69 expression upregulate the Ly49D and Ly49G2 receptors, responsible for activating B cells and NK cells and whether this would lead to successful aGVHD prevention.

**Results:** In a cohort of 180 NDMM patients, median OS (mOS) was 29 and 43 months for cases with (w) or without (w/o) 1q (P=0.038), among which 24.3, 43.3, and 43.8 months for 1q copy number ≥3, =3, and =2 (P=0.030), respectively; whereas Btz-based therapy displayed a marked increase in response rate ≥VGPR, it failed to improve mOS of 1q patients significantly (28.5 and 33.9 months for patients w or w/o Btz treatment, P=0.983); in contrast, Btz treatment dramatically prolonged OS in patients w/o w 1q (53.7 and 28.5 months, P=0.016). To explore the molecular basis for the adverse effect of 1q on prognosis, expression of the 1q21 genes related to drug resistance was examined. Notably, robust expression of HIF-1ß at protein level was found in 1q+ MM cells, while no difference observed in CKS1B, a biomarker widely used for 1q MM, or PSMB4 and MCL-1. Further, analysis of additional 40 1q+ MM cells, while no difference observed in CKS1B, a biomarker widely used for 1q MM, or PSMB4 and MCL-1. Thus, HIF-1ß was employed for extensive phenotyping of WT and CD69-/- NK cells and RNAseq analyses were used to elucidate the molecular mechanisms implicated.

**Results:** CD69-/-mice were highly resistant to aGvHD and significantly more efficient at eliminating hyper-reactive allogenic T cells in vivo. This phenotype was reproduced in WT mice treated with a CD69 neutralizing monoclonal antibody and was not reproduced in CD69-/-HLA-B27 mice. This suggests that CD69 is one of the main potentially fatal complications of this treatment with an incidence as high as 50%. The NK cell population has been extensively studied as a potential target for treatments, as these cells have the capacity to potentiate the graft versus leukemia effect with a minimum risk for graft versus host reactions. Indeed, the abundance of circulating NK cells has been inversely correlated with the probability to develop (aGvHD). CD69 is a C-type lectin expressed on the surface of certain immune cell progenitors as well as activated mature leukocytes. CD69-/- mice were previously shown to eliminate tumour cells more effectively than WT NK cells.

**Aims:** We wished to examine whether CD69-/- NK cells would have a higher cytolytic capacity against activated allogenic NK cells and whether this would lead to successful aGvHD prevention.

**Methods:** We took advantage of a fully allogenic aGvHD mouse model in which wild type (WT) or CD69-/- BALBc mice were lethally irradiated and reconstituted with C57BL6 HSCs and naive T cells. Results were confirmed by in vivo killing assays as well as by use of CD69 neutralizing antibodies. Mouse strains deficient in T cells, B cells and NK cells were used to establish the NK cells as the critical component responsible for the observed phenotype. Mass cytometry was employed for extensive phenotyping of WT and CD69-/- NK cells and RNAseq analyses were used to elucidate the molecular mechanisms implicated.

**Results:** CD69-/- mice were highly resistant to aGvHD and significantly more efficient at eliminating hyper-reactive allogenic T cells in vivo. This phenotype was reproduced in WT mice treated with a CD69 neutralizing monoclonal antibody and was not reproduced in CD69-/-HLA-B27 mice. This suggests that CD69 is one of the main potentially fatal complications of this treatment with an incidence as high as 50%. The NK cell population has been extensively studied as a potential target for treatments, as these cells have the capacity to potentiate the graft versus leukemia effect with a minimum risk for graft versus host reactions. Indeed, the abundance of circulating NK cells has been inversely correlated with the probability to develop (aGvHD). CD69 is a C-type lectin expressed on the surface of certain immune cell progenitors as well as activated mature leukocytes. CD69-/- mice were previously shown to eliminate tumour cells more effectively than WT NK cells.

**Summary/Conclusions:** NK cells treated with anti-CD69 mAb show a higher capacity to eliminate hyper-reactive allogenic T cells and confer resistance to aGvHD. This data could pave the way for novel therapeutic strategies to optimize allogenic HSCT.

**LB2604**

**GLOBAL PHASE 2 TRIAL OF THE CD19-TARGETED THERAPY CTL-019 IN ADULT PATIENTS WITH RECURRENT OR TRANSFUSORY DIFFUSE LARGE B-CELL LYMPHOMA – AN INTERIM ANALYSIS**


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\textbf{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.

\textbf{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.

\textbf{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 and had disease progression after or were ineligible for autologous hematopoietic cell transplantation (AHCT). Autologous T-cells were transduced via a lentiviral vector encoding an anti-CD19 CAR, expanded, cryopreserved, shipped, and infused at study sites. The primary endpoint (centrally reviewed by an independent review committee) was best overall response rate (ORR: CR + partial response [PR]).

\textbf{Results:} 141 pts were treated. Following restaging, bridging therapy, and lymphodepleting chemotherapy (fludarabine 25 mg/m2/cycle; cyclophosphamide 250 mg/m2/day + 3 d or bendamustine 90 mg/m2/day + 2 d), 85 pts received a single dose of CTL019 transduced cells (median, 3.1 ± 10^8 [range, 0.1–10^10] cells). Median time from infusion to data cutoff (20 December 2016) was 7.0 mo (range, 0.2–29.9 mo).

\textbf{Conclusion:} Among 51 pts with ≥3 mo follow-up or earlier discontinuation, best ORR was 59% (95% CI, 44% to 72%), and 37% had PR; the primary endpoint was met. CR and PR rates at 3 mo were 37% and 8%, respectively. All pts in CR at 3 mo maintained full activity at data cutoff. Among 43 pts, 19 (44%) with a median follow-up of 17.2 mo had a durable first response (median duration of response was not reached). CTL019 was detectable in peripheral blood by quantitative PCR for up to 355 days in responders. Cytokine release syndrome (CRS) was graded using the Penn scale and managed by a protocol-specific algorithm. Among 123 pts with ≥3 mo follow-up or earlier discontinuation, best ORR was 95% (95% CI, 89% to 98%) with 65% CR and 30% PR; the primary endpoint was met. CR and PR rates at 3 mo were 71% and 19%, respectively. All pts in CR at 3 mo maintained full activity at data cutoff. Among 32 pts, 19 (59%) with a median follow-up of 17.2 mo had a durable first response (median duration of response was not reached). Among 51 pts with ≥3 mo follow-up or earlier discontinuation, best ORR was 95% (95% CI, 89% to 98%) with 65% CR and 30% PR; the primary endpoint was met. CR and PR rates at 3 mo were 71% and 19%, respectively. All pts in CR at 3 mo maintained full activity at data cutoff. Among 32 pts, 19 (59%) with a median follow-up of 17.2 mo had a durable first response (median duration of response was not reached). No deaths were attributed to CTL019.

\textbf{Summary/Conclusions:} This planned interim analysis of a global study of CTL019 in adults with R/R DLBCL confirms the high response rates and durable CRs observed in the previous single-center experience in a cohort of highly pretreated patients. Centralized manufacturing was feasible. CTL019 was generally well tolerated without instance of treatment-related mortality. CRS and other AEs were effectively and reproducibly managed by appropriately trained investigators.
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